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Persistent Organic Pollutants in Human Milk

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This work was supported by the Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg (State Institute for Chemical and Veterinary Analysis of Food) based on a Project Cooperation Agreement with the United Nations Environment Programme (UNEP), funded by the Global Environment Facility (GEF 4894, 4886, 6978 and 4881).

ISBN 978-3-031-34085-7 ISBN 978-3-031-34087-1 (eBook)
https://doi.org/10.1007/978-3-031-34087-1

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

We currently live in a world containing a lot of manmade chemicals spread around us. While many of them contributed to the prosperity and economic growth and made our life more comfortable since the second half of the 20th century, some chemicals persist, exhibit undesired effects on quality and status of the environment, contaminate food chains, and negatively affect humans.

For decades, human milk has been used as indicator for the overall human exposure of the general population to persistent organic pollutants (POPs). It has been shown that levels of various lipophilic POPs in human milk are representative of levels in plasma, serum, and adipose tissue. Breast-fed infants are exposed to higher intakes of these compounds than adults, although for a small proportion of their lifespan. Therefore, monitoring of human milk has been important not only as indication of the bioaccumulation in humans but also with regard to possible health effects for breast-fed infants.

In the mid-1980s, the World Health Organization's (WHO) Regional Office for Europe started a comprehensive programme on possible health risks of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF). It concentrated particularly on the health risk of infants due to exposure through contaminants in human milk, with the aim of preventing and controlling exposure to these environmental chemicals. Between 2000 and 2019, WHO and the United Nations Environment Programme (UNEP) performed several rounds of global surveys on levels and trends of POPs in human milk with a gradually increasing set of chemicals.

Once the risks from POPs contamination became known and globally recognized, we had negotiated a global environmental instrument, the Stockholm Convention on Persistent Organic Pollutants to eliminate these chemicals from production and use and thus reduce environment contamination and minimize human exposure. Monitoring programs and activities were set up within the Convention to provide the global community with information on the effectiveness of measures put in place.

I see this complex review covering in detail decades of work by experts as an opportunity to show the readers unique outcomes. The UNEP/WHO milk surveys data show the success of cooperation between various international organizations and people over long time and confirm gradual elimination of POPs from our environment.

WHO and UNEP hereby jointly acknowledge funding for the survey rounds provided by the Global Environment Facility (GEF), the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden, the European Union's Global Public Goods and Challenges Programme (GPGC), and certain participating countries. We are also grateful for the continued support to the monitoring activities coming from the national coordinators, laboratory and administrative staff, and all participating mothers. Last but not least, we thank the team of experts and authors and appreciate a long-term fruitful collaboration with the State Institute for Chemical and Veterinary Analysis of Food (Chemisches und Veterinäruntersuchungsamt, CVUA), Freiburg, Germany.

United Nations Environment Programme (UNEP), Economy Division, Chemicals and Health Branch
Geneva, Switzerland February 2023

Jaqueline Alvarez

Preface

In the 1980s and 1990s, two rounds of human milk surveys were conducted by the World Health Organization (WHO) on concentrations of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF). Between 2000 and 2019, WHO and the United Nations Environment Programme (UNEP) performed five rounds of global surveys on concentrations and trends of persistent organic pollutants (POPs) in human milk, partly as joint studies, with participation of 82 countries.

During this period, the number of analytes of interest increased from initially focusing only on PCB, PCDD, and PCDF via the initial twelve POPs listed in 2001 at signing of the Stockholm Convention on POPs to currently 30 chemicals after numerous amendments by the Conference of Parties until 2019, from these 28 chlorinated or brominated and 2 fluorinated. A guidance document for the Global Monitoring Plan recommends the monitoring of human milk as indicator for human exposure as a key element for control of the effectiveness of the convention.

The human milk surveys in the past decades have generated valuable results to support the effectiveness evaluation of the Stockholm Convention. However, the presentation of the concept, analysis, results, and discussion of these global studies for these POPs is a complex task which cannot be fulfilled in one publication. Therefore, a series of publications was prepared with various aspects and allocated according to the specifically addressed aspects as chapters to five parts of this compendium "Persistent organic pollutants in human milk".

- Part I, the introduction, provides an overview on human milk surveys on POPs from a historical perspective, followed by the overview of the WHO/UNEP-coordinated exposure studies on POPs in human milk performed between 1987 and 2019 and their link to the Stockholm Convention. It also includes an article on the Stockholm Convention and its implementation by regional and global monitoring reports.
- Part II shows the analytical methods and the long-term quality control for determination of chlorinated and brominated compounds at the WHO- and UNEP-coordinated human milk studies 2000–2019.
- Part III presents the results and discussion for five groups of chlorinated and brominated compounds: (1) PCB, PCDD, and PCDF; (2) chlorinated pesticides

and industrial chemicals; (3) polybrominated substances; (4) chlorinated paraffins; and (5) polychlorinated naphthalenes.
- Part IV assesses time trends derived from countries with repeated participation for PCB, PCDD, and PCDF, for selected chlorinated pesticides and for the listed perfluoroalkyl substances. Furthermore, a chapter describes a risk–benefit analysis for the breast-fed infant regarding dioxin-like compounds.
- Part V summarizes the study programme, presents key messages, and looks at upcoming needs and challenges.

We acknowledge the contribution and support from WHO and UNEP, the national coordinators of the jointly coordinated exposure studies, assisted by the respective health, laboratory and administrative staff, from all authors and the publisher—and last but not least, all mothers providing human milk.

This special issue with publication on open accessible channels and in printed forms was developed based on a Project Cooperation Agreement between UNEP and CVUA Freiburg in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region under the Stockholm Convention" (GEF ID 4894) and "Continuing Regional Support for the POPs Global Monitoring Plan under the Stockholm Convention" in the Africa, Pacific, and Latin-American and Caribbean Region (GEF ID 4886, 6978 and 4881, respectively), funded by the Global Environment Facility (GEF) and in close collaboration with and support of CVUA Freiburg.

Freiburg, Germany — Rainer Malisch
Münster, Germany — Peter Fürst
Brno, Czech Republic — Kateřina Šebková
February 2023

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About the Editors and Contributors

About the Editors

Rainer Malisch, State-examined food chemist. After finishing his doctoral thesis on environmental contamination by organochlorine pesticides, polychlorinated biphenyls, and phthalates at the University of Münster (1981), Dr Rainer Malisch worked for the CVUA Freiburg (in 2000 so renamed), initially as head of the residue laboratory for veterinary drugs, later for dioxins and PCB. In 1993, he was appointed head of the residue department. He supported the WHO/UNEP-coordinated exposure studies as head of the reference laboratory for determination of chlorinated and brominated POPs in human milk samples of the period 2000–2019. After designation of two Community Reference Laboratories (CRLs) to CVUA Freiburg in 2006, he became director of the CRL for Pesticides Residues in Food of Animal Origin and Commodities with High Fat Content (with this responsibility until 2010) and of the CRL for Dioxins and PCBs in Feed and Food. In 2010, the CRLs were renamed into "European Union Reference Laboratory" (EURL). In 2018, the tasks of the EURL for dioxins and PCBs were extended to all halogenated POPs ("EU Reference Laboratory for halogenated persistent organic pollutants (POPs) in Feed and Food"). He was member of several committees and commissions and retired in 2019. State Institute for Chemical and Veterinary Analysis of Food (Chemisches und Veterinäruntersuchungsamt, CVUA), Freiburg, Germany

Peter Fürst studied Food Chemistry at the Universität of Münster/Germany where he received his PhD in 1982. From 1981 until his retirement in 2019, he joined the Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (MEL) and its legacy institutes where he held various positions. Since 2014, he was Chief Executive Officer of the institute which is a public-law institution. In 1988, he took a six-month sabbatical leave at the Centers for Disease Control, Atlanta/USA and Health and Welfare, Ottawa/Canada. His main working areas are the investigation and assessment of organic contaminants in food, feed, and human milk. He is a member of several European committees and commissions, including the Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) from 2006–2015. In 2009, he was appointed Honorary Professor at the Chemical Faculty of the University of Münster where he gives lectures since 1995 on

"Chemistry and Analysis of Pesticides and Contaminants" for students at the Institute of Food Chemistry.
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Kateřina Šebková has a degree and PhD in chemistry (graduate of Institute of Chemical Technology in Prague, Czech Republic and Université Louis Pasteur-Centre National de Recherches Scientifiques in Strasbourg, France). She worked as chemical expert and negotiator for the Czech Ministry of Environment in chemicals management, in particular on mercury and persistent organic pollutants, at the EU and the United Nations level for eight years. She joined the RECETOX, Faculty of Science at the Masaryk University in Brno, Czech Republic in 2012. Currently, she is director of the National Centre of Toxic Compounds and of the Stockholm Convention Regional Centre for capacity building and the Transfer of Technology, two bodies that are hosted by RECETOX. Her work comprises cooperation with Czech and international stakeholders, teaching, training, and support policy-science communication and application of science in practice and building expert capacities. She also works with international organizations such as UN Environment, World Health Organization, UNIDO, UNDP, and Secretariats of the multilateral environmental agreements protecting human health and environment as consultant, trainer, and chairperson. Last but not least, she is coordinating the POPs monitoring in the Central and Eastern European Region within the Stockholm Convention on POPs, chaired expert groups for effectiveness evaluation and monitoring under the Minamata Convention on Mercury, and is a member of the Technical advisory group on chemicals, waste and pollution for Executive Director at UNEP and a member of the High-Level Roundtable on the EU Chemicals Strategy for Sustainability.
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About the Authors

Jacqueline Alvarez is the Chief of Chemicals and Health Branch in the United Nations Environment Programme Economy Division. She has 30 years of political, management, and technical expertise, including experience of 14 years with the Government of Uruguay, where she led negotiations on behalf of her region in multiple occasions. At UNEP, she has held various roles including UNEP Regional Director and Representative for the Latin America and the Caribbean region. She was the first Regional Sub-Programme Coordinator for Chemicals and Waste for the Latin America and Caribbean region, after which she moved to the Basel, Rotterdam and Stockholm Conventions Secretariat. She was the Head of the Knowledge and Risk Unit, where under her leadership, the science-policy-action nexus on chemicals and waste significantly grew while bringing in critical partners such as governments, civil society, and private sector. Jacqueline is a chemist by training, specialized in pharmacy, and has worked over her career on national, regional, and international programmes.

United Nations Environment Programme (UNEP), Economy Division, Chemicals and Health Branch, Châtelaine, GE, Switzerland

Benjamin Dambacher studied food chemistry at the University of Hohenheim. His diploma thesis dealt with the generation and analysis of mixed chlorinated/brominated homologs of halogenated natural products. From 2013 to 2021, he was head of a laboratory in the area of pesticide residues and organic contaminants at the State Institute for Chemical and Veterinary Analysis of Food (CVUA) Freiburg. During this time, among many other tasks, he was responsible for the determination of these analytes in the human milk samples of the WHO/UNEP-coordinated exposure studies. Since November 2022, he has taken over the task as head of the department comprising the mycotoxin laboratory and LC-MS/MS determination at CVUA Sigmaringen.
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Jerzy Falandysz, habilitated food toxicologist, studied pharmacy (Master's degree), defended his doctorate in food science, and then obtained a postdoctoral degree in food toxicology at the Faculty of Pharmacy of the Medical Academy in Gdańsk. He started working at the Department of Food Sciences of the Faculty of Pharmacy of the Medical University of Gdańsk (technical assistant, 1971), and then at the Food Research Laboratory of the Port Health Office in Gdynia (assistant, 1972–1979); Department of Veterinary Toxicology and Food Hygiene (head) of the Veterinary Hygiene Research Station in Gdańsk (assistant, assistant professor and associate professor, 1980–1991); Department of Environmental Chemistry and Ecotoxicology (head) of the Faculty of Chemistry, University of Gdańsk (full professor, 1991–2018); Department of Toxicology at the Faculty of Pharmacy of the Medical University of Lodz (since 2021) in Poland. Meanwhile, Prof. Falandysz was a long-term visiting professor at Ehime University in Matsuyama, Japan (1990–1991 and 2000), the University of Umeå in Sweden (1995–1996), the Institute of Mountain Hazards and Environment in Chengdu, China (2010 and 2012) and the University of Cartagena in Colombia and Medicinal Plants Research Institute, Yunnan Academy of Agricultural Sciences in Kunming, China (2015–2021). He is also involved as an expert at the European Food Safety Authority (EFSA). His research interests focus on food chemistry and toxicology, environment and analytics with particular emphasis on persistent organic pollutants, metallic elements, metalloids, and radionuclides.
Department of Toxicology, Medical University of Lodz, Lódź, Poland

Alwyn Fernandes is a chartered scientist with more than 35 years of research experience in environmental and food safety with respect to chemical contaminants and their impact on human health and ecology. He is an internationally recognized expert in this field and has collaborated with a number of universities and research

institutions across Europe. He is currently an invited research fellow at the University of East Anglia and at the EU Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food (EURL POPs) in Freiburg, Germany, where he chairs a working group on brominated and chlorinated contaminants in feed and food. Earlier, he led research on emerging contaminants and food safety at the UK Food and Environment Research Agency, as a senior scientist until his retirement in 2017. He is widely published with a multidisciplinary output covering topics ranging from dietary exposure of humans and animals to environmental contaminant, to PTEs and radioactivity in edible fungi. He has also edited a number of dedicated journal issues and books on these topics.
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Björn Hardebusch, State-examined food chemist. Björn Hardebusch studied food chemistry at the Westfälische-Wilhelms-Universität (WWU) in Münster, Germany, until the first state examination in 2001 and completed the legal training at the State Institute for Chemical and Veterinary Analysis (CVUA) Münster until his second state examination in 2002. After finishing his PhD studies on biotransformation of terpenes by fungi at the Leibnitz University Hannover in 2006, he worked at a private laboratory for regulatory studies mainly performing analysis of pesticides. In 2007, he switched to the CVUA Freiburg, Germany, to work as a scientist at the European Union Reference Laboratory for Pesticides Residues in Food of Animal Origin and Commodities with High Fat Content (EURL AO). In 2020, he took over the position as Director of the EURL AO.
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Jiří Kalina graduated in discrete mathematics and environmental chemistry from the Masaryk University in Brno, Czech Republic, where he also finished his PhD in environmental chemistry in 2017. After several years in a private company (ECO-management), he works at the Institute of Biostatistics and Analyses (IBA) and the Research Centre for Toxic Compounds in the Environment (RECETOX), both at the Masaryk University. His research focuses on persistent air pollution, in particular the temporal and spatial distribution of pollutants and the relationship with environmental epidemiology and human health. His field of work includes biostatistics, data analysis and mathematical analysis and modelling.
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Jana Klánová is professor of environmental chemistry and director of the RECETOX centre at the Faculty of Science, Masaryk University, Czech Republic. Her field is research on relations between the environment and human health. She coordinates the new pan-European infrastructure (ESFRI) EIRENE on human exposome as well as the GEO initiative GOS4POPs (Global Observation System for Persistent Organic Pollutants). She is member of the Czech Council for research infrastructures and Scientific Advisory Board of the Czech Technology Agency. She is the principal investigator of dozens of large European research projects and

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Karin Kypke, State-examined food chemist. Dr. Karin Kypke studied food chemistry at the University of Stuttgart and graduated with the first and second state examinations. At the University of Hohenheim, she did her PhD thesis in organic chemistry on the extraction and structure determination of natural substances in the bark and leaves of Toona sureni (Meliaceae), which act as insect feeding deterrents. Since 1980, she worked at the State Institute for Chemical and Veterinary Analyses of Food, where she headed the laboratory for residue analysis of pesticides and organic contaminants in food of animal origin and human milk, accredited in 1998. Since 2006, she was also responsible for tasks of the European Union Reference Laboratory for Pesticide Residues in Food of Animal Origin and Commodities with High Fat Content (EURL AO). From 2000 to 2014, organochlorine pesticides and certain chlorinated and brominated POPs were determined in human milk samples under her leadership as part of the WHO/UNEP-coordinated exposure studies. She retired in 2014.
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Ralf Lippold, a state-certified food chemist, joined the Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg in 1988. His initial working area as head of a laboratory comprised food additives, species differentiation, and polycyclic aromatic hydrocarbons. In 1990, he moved internally to the Department of Residues and Contaminants. There he worked on residues of veterinary drugs and pesticides as well as organic halogenated contaminants. In 2012, he became head of

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Karin Malisch studied biology with a focus on microbiology at the University of Münster, where she received her PhD in immunology in 1982. Afterwards she worked as a laboratory manager for serology and bacteriology and as a freelancer. From 2006 to 2019, she organized the international scientific workshops of the two EU Reference Laboratories at CVUA Freiburg. From 2009 to 2019, she was responsible for organizing the WHO/UNEP studies on human milk at CVUA Freiburg. In cooperation with UNEP, she coordinated all sample management issues with the national coordinators. Furthermore, she was substantially involved as author in the preparation of the Guidelines for Organization, Sampling and Analysis of Human Milk on Persistent Organic Pollutants.
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Gamini Manuweera is an Eco-Toxicological Chemist, graduated from the University of Missouri-Columbia, USA. His area of research focused on sampling devices for determining the presence, bioavailability, and concentration of non-polar organic contaminants in aquatic systems. He holds a US patent related to his doctoral research and also holds several Presidential awards from Sri Lanka related to research on chemicals management. Dr. Manuweera has supervised several post graduate studies in analytical and environmental chemistry and a teacher of environmental and toxicological chemistry and of chemical regulatory science courses at post graduate level. He has served in the United Nations Environment Programme at the Secretariat of Basel, Rotterdam, and Stockholm Conventions and in the Chemicals and Health Branch until his retirement in 2022. One of his tasks from 2018 to 2022 was to coordinate the human milk study performed until 2019 including follow-up, e.g. by multiple reports. He is also a member of number of international expert bodies on chemicals management.
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Gerald Moy received his doctorate in physical organic chemistry from Oregon State University and after a post-doc at the University of New Mexico, began his career with the US Food and Drug Administration in Washington, D.C. in 1976. He joined the World Health Organization (WHO) in 1987 as the Regional Food Safety Adviser for the WHO Western Pacific Region based in Kuala Lumper, Malaysia. In 1991, he transferred to WHO Headquarters in Geneva, Switzerland to become the GEMS/Food Manager responsible for exposure assessment of chemicals in food, including human milk. In this regard, he co-authored a book on total diet studies and has promoted their adoption by countries through a series of international and

regional conferences. Since his retirement from WHO in 2008, he has served as a food safety consultant to various governments, international organizations, and the food industry and continues to publish articles on various food safety topics.
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Richard E. Peterson is an emeritus Professor in Toxicology at the University of Wisconsin-Madison. He received his BS degree (1967) from the University of Wisconsin-Madison and his PhD degree in pharmacology (1972) from the Marquette University School of Medicine (now the Medical College of Wisconsin). He joined the faculties of the School of Pharmacy and the Environmental Toxicology Center at the University of Wisconsin-Madison in 1975. He currently serves as Deputy Director of the NIEHS Center for Developmental and Molecular Toxicology. His decades of research is in a broad range of toxicological areas with special emphasis on the toxicity of dioxin-like compounds and the function of the Ah-receptor. He has also been globally involved with human and environmental risk assessment of dioxin-like compounds and other persistent organic pollutants.
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Julia Polley studied food chemistry at the Karlsruhe Institute of Technology (KIT) and completed her diploma thesis at the Department of Food Chemistry and Toxicology in 2014 on the topic of "Determination of the uptake of nickel and nickel oxide nanoparticles in bronchial lung cells Beas-2B using Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)". Since 2018, she has been working as a State-certified food chemist at the Chemisches und Veterinäruntersuchungsamt Freiburg in the department "residues and contaminants" and as a quality manager. This includes the support of the European Union Reference Laboratory for Pesticides in Food of Animal Origin and Commodities with High Fat Content (EURL AO) as a research assistant.
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Daria Sapunova graduated from a five-year specialization study program in ecology and environmental protection at Tomsk State University of Control Systems and Radioelectronics in Russia in 2012. After the graduation she worked as an environmental coordinator in a private sector. Since 2021, Daria S. has been doing her PhD at RECETOX on the topic "An impact of ambient air pollution on human diseases" in the doctoral degree program Environmental Health Sciences. At the same time, she has been working at RECETOX as a researcher and participating in science projects of the institute.
RECETOX, Masaryk University, Brno, Czech Republic

Alexander Schächtele, State-examined food chemist. Dr. Alexander Schächtele studied food chemistry at the Technical University Munich (TUM) and completed

his legal training at the State Institute for Chemical and Veterinary Analysis (CVUA) Freiburg in 2003. He finished his PhD studies on ”Determination of brominated and brominated-chlorinated contaminants in biological matrices using HRGC/HRMS” with Prof. Michael Oehme at the Organic-Analytical Institute of the University of Basel, Switzerland, in 2006. Afterwards he worked for the Community Reference Laboratory (CRL; since 2010 “EU Reference Laboratory [EURL]”) for Dioxins and PCBs in Feed and Food located at CVUA Freiburg as head of the GC-MS unit. In 2018, the tasks of this EURL were extended to all halogenated POPs. In 2019, he became director of this “EU Reference Laboratory for halogenated persistent organic pollutants (POPs) in Feed and Food”. Additionally, he was named head of the department for analysis of pesticides and contaminants at CVUA Freiburg in 2020.
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Angelika Tritscher graduated in food chemistry and got her PhD in biochemical toxicology, both at the University of Würzburg, Germany. She worked from 2003 to 2019 at the World Health Organization's headquarter in Geneva, Switzerland, first in the International Programme on Chemical Safety and then in the Department of Food Safety and Zoonoses. As a food toxicologist and risk assessor, her responsibilities included serving as WHO expert on chemical exposures and health risk, in particular leading the scientific advice work as basis for international food standards, Codex Standards. In her capacity as WHO Joint Secretary to JECFA and JMPR, Dr Tritscher was responsible for the organization of these independent international expert meetings, assuring sound science-based assessments and presentation of the outcome of this work in international fora, in particular Codex negotiations. From 2009 to 2011, the coordination of the human milk studies on levels of POPs in close cooperation with UNEP was under her responsibility.
Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland

Karin Tschiggfrei studied food chemistry (Bachelor and Master of Science) at the Ludwig-Maximilian University of Würzburg. She completed her education as a state-certified food chemist after a temporary appointment in the office of a member of the German Bundestag (committee of food and agriculture). Since 2018, she has been working in the “European Union Reference Laboratory for Pesticide Residues in Food of Animal Origin and Commodities with high Fat Content” (EURL AO) and the “European Union Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food” (EURL POPs) located at the State Institute for Chemical and Veterinary Analysis (CVUA) Freiburg. Her research focuses include the analysis of polychlorinated naphthalenes in food and human milk.
State Institute for Chemical and Veterinary Analysis of Food (Chemisches und Veterinäruntersuchungsamt, CVUA), Freiburg, Germany

Martin van den Berg is an Emeritus Professor in Toxicology, former deputy director of the Institute of Risk Assessment Sciences of the University of Utrecht, and a European Registered Toxicologist (ERT). He is an honorary professor in environmental toxicology at the University of Queensland (Brisbane) and a visiting professor at the Royal Chulabhorn Research Institute and Graduate School in Bangkok. In 2006, he received an honorary doctorate from the University of Umea, Sweden, for his research on mixture toxicity of dioxin-like compounds. He has published over approximately 375 peer-reviewed scientific articles, short papers, and conference proceedings. Prof. van den Berg has been and is an advisor to many national and international organizations (Dutch National Health Council, WHO, FAO, IARC, EU, and US) involved with (eco)toxicological risk assessment of environmental and food contaminants and pesticides.
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Majorie van Duursen is professor of Environmental Health and Toxicology at the Vrije Universiteit Amsterdam (VUA). She is director of the BSc programme Health & Life Sciences at VUA. As principal investigator, she has been/is involved in various (inter)national projects to improve toxicological risk assessment of environmental pollutants, including dioxins, endocrine disruptors, PFAS, and microplastics. Prof van Duursen is coordinator of the H2020 EU-funded project FREIA (www.freiaproject.eu) that aims to improve test methods for identification of endocrine disruptors that affect female reproductive health, to be used in regulatory risk assessment. She is European Registered Toxicologist and chairs and participates in various national and international organizations and committees related to environmental health issues; she is chair of the standing subcommittee Classification of Substances Toxic to Reproduction and Development, of the Health Council of the Netherlands and member of expert groups at the European Chemical Agency (ECHA) and European Food and Safety Authority (EFSA). She is also member of the editorial board of Toxicological Sciences and Regulatory Toxicology and Pharmacology and she is member of the Dutch and US Societies of Toxicology and the European Society of Endocrinology.
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F. X. Rolaf van Leeuwen worked at the Bilthoven Division of the European Centre for Environment and Health of the World Health Organization (WHO) in the period 1995–2001. The office of the Bilthoven Division was located on the premises of the National Institute for Public Health and the Environment, Bilthoven, The Netherlands. Besides his activities for the WHO Air Quality Guidelines for Europe, Dr van Leeuwen organized, in collaboration with the International Programme on Chemical Safety, the international expert meeting on the Assessment of the health risk of dioxins: re-evaluation of the tolerable daily intake (1998), and was responsible for the coordination of the Third Round of the WHO-coordinated exposure study on Levels of PCBs, PCDDs and PCDFs in human milk, and for the Fourth Round of

the WHO-coordinated Interlaboratory Quality Assessment of Levels of PCBs, PCDDs, and PCDFs in Human Milk and Blood Plasma. The latter study resulted in the selection of the reference laboratory for the 3rd round of the WHO-coordinated exposure study. In the years after his employment at WHO he was occasionally involved in follow-up activities related to this exposure study.
World Health Organization (WHO), European Centre for the Environment and Health at the National Institute of Public Health and the Environment, Bilthoven, The Netherlands

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List of Abbreviations and Acronyms

BDE	Brominated diphenyl ether
CEE	Central and Eastern Europe (alternatively used: EEG)
COP	Conference of the Parties
CP	Chlorinated paraffin(s)
CV	Coefficient of variation
CVUA	Chemisches und Veterinäruntersuchungsamt (State Institute for Chemical and Veterinary Analysis of Food)
DDD	Dichlorodiphenyldichloroethane (metabolite of DDT)
DDT	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethylene (metabolite of DDT)
DDT complex	Sum of all analytes (o,p′-DDT, p,p′-DDT, o,p′-DDT, p,p′-DDE, o,p′-DDD and p,p′-DDD) detected, calculated as DDT
DLC	Dioxin-like compounds
DL-PCB	Dioxin-like polychlorinated biphenyl(s)
DL-POPs	Dioxin-like persistent organic pollutants (including 7 PCDD, 10 PCDF, and 12 DL-PCB with assigned TEF)
EEG	Eastern European Group (alternatively used: CEE)
EHEN	European Human Exposome Network
EIRENE	Environmental Exposure Assessment Research Infrastructure
ESFRI	European Strategy Forum on Research Infrastructures
EU	European Union
EURL	European Union Reference Laboratory
EURL POPs	EURL for halogenated persistent organic pollutants in feed and food
GC	Gas chromatograph(y)
GC/ECD	Gas chromatography with electron capture detection
GC/HRMS	Gas chromatography with high resolution mass spectrometric detection

GC/MS	Gas chromatography with mass spectrometric detection
GEF	Global Environment Facility
GMP	Global Monitoring Plan
GPC	Gel permeation chromatography
GRULAC	Group of Latin American and Caribbean Countries
HBCDD	Hexabromocyclododecane(s)
HBGV	Health-Based Guidance Values
HBM4EU	European Human Biomonitoring Initiative
HCB	Hexachlorobenzene
HCBD	Hexachlorobutadiene
HCH	Hexachlorocylohexane(s)
HPLC	High performance liquid chromatograph(y)
HRGC	High resolution gas chromatography
HRMS	High resolution mass spectrometry
HxBB	Hexabromobiphenyl
Indicator PCB	Sum of six NDL-PCB (ΣPCB_6) including the congeners PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180 (also called: marker PCB)
LB	Lower bound
LC	Liquid chromatography
LCCP	Long-chain chlorinated paraffin(s)
LCL	Lower control level (*of quality control charts*)
LOD	Limit of detection
LOQ	Limit of quantification
LWL	Lower warning level (*of quality control charts*)
Marker PCB	See Indicator PCB
MCCP	Medium-chain chlorinated paraffin(s)
MS	Mass spectrometry or mass spectrometer
MS/MS	Tandem mass spectrometry
NA	Not applicable
ND	Not detected
NDL-PCB	Non-dioxin-like polychlorinated biphenyl(s)
OC	Organochlorine
OCP	Organochlorine pesticide(s)
PBB	Polybrominated biphenyl(s)
PBDE	Polybrominated diphenyl ether(s)
$\sum PBDE_6$	Sum of 6 PBDE (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-175/183)
$\sum PBDE_7$	Sum of $\sum PBDE_6$ plus BDE-209
PCA	Pentachloroanisol
PCB	Polychlorinated biphenyl(s)
ΣPCB_6	Sum of six NDL-PCB, see Indicator PCB
PCDD	Polychlorinated dibenzo-p-dioxin(s)
PCDF	Polychlorinated dibenzofuran(s)

PCN	Polychlorinated naphthalene(s)
PCP	Pentachlorophenol
PeCB	Pentachlorobenzene
PFAS	Per- and polyfluorinated alkyl substance(s)
PFHxS	Perfluorohexanesulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
POPs	Persistent organic pollutants
PT	Proficiency test
QA/QC	Quality assurance/quality control
REP	Relative effect potency
SC	Stockholm Convention
SCCP	Short-chain chlorinated paraffin(s)
TCDD	Tetrachlorodibenzo-*p*-dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalent(s)
UB	Upper bound
UCL	Upper control level (*of quality control charts*)
UN	United Nations
UNEP	United Nations Environment Programme
USA	United States of America
UWL	Upper warning level (*of quality control charts*)
vLLCP	Very long chain chlorinated paraffin(s)
WEOG	Western European and Others Group
WHO	World Health Organization
WHO-PCB-TEQ	Sum of toxic equivalents of DL-PCB calculated with WHO-TEF
WHO-PCDD/PCDF-TEQ	Sum of toxic equivalents of PCDD/PCDF calculated with WHO-TEF
WHO-TEF	Toxic equivalency factors assigned by WHO assessments (used in this assessment for calculation of results: factors of re-evaluation in 2005)
WHO-TEQ	Total sum of toxic equivalents of mixtures of PCDD/PCDF and DL-PCBs ("WHO-PCDD/PCDF-PCB-TEQ"; "WHO_{2005}-TEQ") calculated with WHO-TEF

Part I

Introduction

Human Milk Surveys on Persistent Organic Pollutants from a Historical Perspective

Peter Fürst

Abstract

Persistent organic pollutants (POPs) were identified in humans who have not been dealing with these chemicals intentionally—from organochlorine pesticides towards industrial chemicals, brominated and fluorine containing POPs. This chapter provides a brief overview of major developments in POPs monitoring in human milk and depicts a gradual broadening of the knowledge underpinned by advances in the instrumentation for chemical analysis as well as expansion of range of analytes that warranted attention. The chapter also shows how, in the course of the past 70 years, human milk monitoring has become an efficient and cost-effective non-invasive biomonitoring tool to evaluate the internal human exposure to POPs and the resulting body burden.

Keywords

Human milk survey · Organochlorine pesticide · Polychlorinated biphenyl · Polychlorinated dibenzo-*p*-dioxin · Polychlorinated dibenzofuran · Brominated flame retardant · Hexabromocyclododecane · Chlorinated paraffin · PFAS · Global distribution · Influence on levels · Temporal trend · Lessons learned

1 Introduction

Over the past 70 years, human milk has become an efficient and cost-effective non-invasive biomonitoring tool to evaluate the internal human exposure with persistent organic pollutants (POPs). Since the first finding of DDT in human milk in 1951, the constant improvement of analytical instrumentation, in particular in

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_1

terms of separation power and sensitivity, enabled the determination of an increasing number of lipophilic and persistent chemicals in human fat tissues and human milk, and thus broadened the knowledge on human body burden with these compounds. Comprehensive monitoring programmes did not only investigate the global distribution and the parameters that have major impacts on the extent of body burden but also examined temporal trends in order to monitor whether the numerous national and international measures taken to reduce the human body burden with POPs show positive effects. This chapter gives an overview on the gradually increasing number of POPs that are analysed in human milk from a historical perspective. Special focus is put on those human milk surveys that have been milestones in initiating global efforts to investigate the spatial contamination with the respective POPs. Moreover, it shortly summarizes important parameters that have a potential impact on the contaminant concentration in human milk, and the requirements for a reliable comparison of POPs concentration in human milk.

2 POPs Analysed in Human Milk in the Course of Time

This chapter provides a brief overview on the gradually increasing number of persistent organic pollutants that were determined in human milk in the course of the past 70 years. Special focus is put on those POPs that were early identified in human milk and subsequently initiated extensive surveys on their occurrence in human milk. It also describes how the advances in analytical instrumentation enabled a better insight into the evaluation of the human exposure to POPs and resulting body burden.

2.1 Organochlorine Pesticides

Organochlorine pesticides of the first generation were found in human milk in some cases already as early as in the 1950s/1960s, such as DDT, hexachlorocyclohexane (HCH), chlordane, and others. They are now listed among the initial 12 POPs of the Stockholm Convention.

The discovery of the insecticidal properties of **DDT** in the late 1930s by Paul Müller was a milestone in the control of pests, such as malaria, typhus, and other insect-borne human diseases. DDT has also found widespread application on crops and livestock production. However, it soon turned out that the stability and long-lasting effects, originally considered as benefits were actually severe drawbacks as DDT was only poorly biodegradable, persistent, accumulated in the food chain and consequently was stored in the human body. Already in 1948, Howell reported a concentration of 17 ppm[1] in body fat of a person who was involved in DDT spaying for 4 years and "had consumed foods containing appreciable quantities of DDT"

[1] In earlier publications, the concentrations are reported as "ppm" rather than "mg/kg" or "mg/L".

(Howell 1948; WHO 1979). After findings of DDT traces in crops, dairy and meat products by the US Food and Drug Administration in 1950, Laug et al. were the first to report on the occurrence of DDT in human fat and human milk samples of subjects who were not employed as pesticide workers (Laug et al. 1951). The survey was intended to set a baseline, i.e. to examine the background contamination of the general population who were not occupationally exposed. The authors analysed 75 samples of abdominal fat obtained at autopsy, biopsy, or abdominal surgery, and 32 human milk samples from women in Washington DC. In 60 out of the 75 abdominal fat samples, and 30 out of 32 human milk samples DDT could be determined. The concentrations were reported as 0–34 ppm (average: 5.3 ppm) and 0–0.77 ppm (average 0.13 ppm) for abdominal fat and human milk, respectively. In contrast to today's convention, the DDT concentrations in human milk in those days were not expressed on a lipid but on a liquid basis. As the patients had never sprayed the insecticide, it was assumed that consumption of food besides inhalation of dust may be an important route of human exposure to DDT. In 1958, Hayes et al. confirmed the special importance of food for exposure of the general population to DDT. From their investigations they concluded that there is strong evidence that fat of animal origin in the diet is the main source from which DDT and its metabolite DDE are absorbed in those subjects who have no particular occupational exposure (Hayes et al. 1958).

The survey by Laug et al. demonstrated the special importance of the contamination of human milk, as it showed that DDT has reached the top of the food chain, i.e. the breastfed baby at a vulnerable period of life (Laug et al. 1951). Their results initiated further investigations into DDT levels in human fat and milk from the general population with no known occupational exposure. These surveys already often comprised besides p,p'-DDT, the major constituent of the technical product, also its isomer o,p'-DDT as well as the metabolite **DDE** (Egan et al. 1965; Quinby et al. 1965). Their concentrations found in human milk in the period between 1950 and the early 1970s were summarized in the Environmental Health Criteria No 9 "DDT and its Derivatives" published by WHO in 1979 (WHO 1979). The data illustrate the widespread distribution of DDT and its metabolites as well as the broad contamination of human milk with this organochlorine pesticide.

Initially, the analysis of DDT was performed by determination of organic chlorine (Carter 1947), colorimetric detection (Prickett et al. 1950), paper chromatography (Evans 1962), and thin-layer chromatography (Knoll and Jayaraman 1973). The introduction of gas chromatography with electron-capture detection (GC-ECD) by Goodwin et al. in 1961 facilitated a substantially improved separation of organochlorine pesticides in human samples (Goodwin et al. 1961). Besides better separation, the GC-ECD determination also enabled considerably lower limits of detection compared to the other analytical approaches applied until then. Nonetheless, higher concentrations of DDT and DDE analysed in human milk by gas chromatography on two columns of different polarity were sometimes still confirmed by paper chromatography (Egan et al. 1965).

The number of organochlorine pesticides analysed in human milk gradually increased over the years. While the first samples generally were only analysed for

DDT and its major metabolite DDE, the improved analytical instrumentation, in particular offered by GC-ECD, enabled to widen the scope of analysis to further organochlorine pesticides that were commercially introduced in the 1940s/1950s because of their insecticidal properties similar to DDT, and then were also found to accumulate to some extent in the human body. This refers inter alia to the DDT analogue **methoxychlor, HCH, chlordane, aldrin, dieldrin,** and **heptachlor**. It was soon shown that aldrin and heptachlor are metabolized in mammals to **dieldrin** and **heptachlor epoxide**, respectively (WHO 1984a, 1989a). This is the reason that dieldrin and heptachlor epoxide rather than aldrin and heptachlor were mainly found in human milk samples. It was not possible to decide whether the occurrence of dieldrin in human milk was the result of the metabolization of aldrin or due to the exposure to the dieldrin pesticide itself.

In 1970, it was reported by Schwemmer et al. that **oxychlordane** is the most relevant mammalian metabolite of chlordane which is a complex technical mixture (Schwemmer et al. 1970). Oxychlordane is more persistent and toxic than the parent compound (WHO 1984b). Consequently, besides trans-**nonachlor**, a constituent of the technical mixture **chlordane**, oxychlordane became the primary analyte to examine the possible contamination of human milk with chlordane. The predominance of the metabolites oxychlordane and also heptachlor epoxide compared to their parent compounds in human milk was reported by Savage et al. in 1981 (Savage et al. 1981).

Comprehensive reports on the widespread regional contamination of the above-mentioned organochlorine pesticides and their metabolites determined in human milk until the 1970s are summarized in the WHO publications Environmental Health Criteria 9 (WHO 1979), 34 (WHO 1984b), 38 (WHO 1984a), and 91 (WHO 1989a).

HCH was first synthesized by Faraday in 1825 (Faraday 1825) and its insecticidal properties were discovered in the 1940s. Since the beginning of commercial production in the early 1950s, HCH became one of the most widely applied insecticides worldwide. Technical HCH mainly consists of five isomers, termed alpha-, beta-, gamma-, delta-, and epsilon-HCH (Hayes and Laws 1991). In older publications, HCH is often misleadingly named benzene hexachloride (BHC) which should not be confused with the fungicide hexachlorobenzene (HCB). Although only present in the technical product at 14–15%, the gamma-isomer was identified to be the active HCH constituent, but with relatively low persistence. In contrast, beta-HCH was found to be the isomer with the highest persistence, followed by alpha-HCH. Their fraction generally made up 7–10% and 65–70% of the technical product, respectively. Alpha- and beta-HCH both have no appreciable insecticidal activity. By purification of the technical product, the gamma-isomer was isolated and commercially marketed as **lindane** in honour to the chemist Van der Linden who described this isolation and purification already in 1912 (Van der Linden 1912). In agriculture, HCH was either applied as lindane or as the technical product, the latter in particular in developing countries. Lindane has also found use for wood and timber protection and in human medicine for treatment of head lice. The global technical HCH usage and its contamination consequences in the environment between 1948 and 1997 is summarized by Li (1999). The HCH pattern in human milk is generally dominated

by the beta-isomer, followed by alpha- and gamma-HCH which are more rapidly metabolized. The ratio between beta-HCH and alpha-/gamma-HCH in human milk gives some indication on the type and phasing out of HCH application (whether technical HCH or lindane). Higher concentrations of alpha-HCH in human milk in addition to beta-HCH point to an application of technical HCH. With increasing time since termination of application, the detected concentration of alpha-HCH decreases. Details on the contamination of human milk by HCH between the 1950s and 1990 showing the pattern and widespread distribution of HCH isomers are given in the publications by WHO in 1991 (WHO 1991a, b).

Findings of **hexachlorobenzene (HCB)** in human fat and human milk were reported for the first time only in 1970 by Acker and Schulte (1970a). The analysis was performed by gas chromatographic separation and mass spectrometric identification (GC-MS). The mean value calculated from the analysis of 43 human milk samples was reported as 5.3 ppm on a lipid basis. This mean value was approximately 50% higher than the sum of DDT and its metabolite DDE. The authors had problems to interpret these findings as they could not imagine that the treatment of seeds, the main application area of this fungicide, could be the source of such high contamination. Several years later it was found that HCB can also be formed in combustion processes and occurs as an industrial waste product in the manufacture of a number of technical products (Courtney 1979). In general, concentrations of HCB in human milk in various countries or regions range widely and appear to be related to the degree of industrialization and/or urbanization within the survey area (WHO 1997).

The findings of organochlorine pesticides in the environment and human matrices as well as the increasing perception of adverse effects in biota and humans led to comprehensive regulations on the production and use of organochlorine pesticides in the 1970s. The regulations on organochlorine pesticides, in particular in the Western World have had positive effects on the extent of contamination in human milk as their concentrations were decreasing after the ban or restrictions (Van Haver et al. 1977; Jensen 1983, 1991; Smith 1999; Fång et al. 2015). However, in countries where DDT and similar lipophilic pesticides were applied longer and in some cases are still applied for malaria vector control today, the decrease observed is not as prominent. Even in developed countries of the Western World, due to their long half-life and persistence, several organochlorine pesticides and metabolites, such as DDE, beta-HCH, and HCB can still be found in human milk more than 40 years after their use was prohibited (Fång et al. 2015; Van den Berg et al. 2017).

2.2 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCB) are complex technical mixtures with a chlorine content between around 30 and 60%. Although 209 different PCB with 1–10 chlorine atoms, termed congeners, are theoretically possible, only up to 130 congeners are likely to occur in the commercial products (WHO 1993). PCB were commercially introduced around 1928 and found broad use in open and closed application.

Determination of PCB in the environment and biota was quite challenging. Although in use since more than 30 years, it was only in 1966 that Jensen identified PCB for the first time in biota (Jensen 1966, 1972). At that time the analyses for organochlorine pesticides were performed by GC-ECD with packed GC columns. Jensen noticed a series of peaks behind DDE in the chromatograms which increased in samples through different levels of the food chain. When analysing a white-tailed eagle that was found dead in the archipelago of Stockholm and contained "enormous" amounts of the unknown compounds, Jensen was able to isolate these unknown compounds by thin-layer chromatography and identified them as PCB by GC-MS (Jensen 1972).

In their investigations of human milk on organochlorine pesticides, Acker and Schulte also noticed unknown peaks eluting behind DDT and assumed that these could be the same which Jensen found in biota. Performing GC on two columns of different polarity, they could identify PCB for the first time in human milk (Acker and Schulte 1970b). The initial analyses for PCB in human milk were hampered by the lack of separation power, as the PCB congeners could only be determined as "humps" in the chromatograms. By analysing different technical PCB mixtures, it was found that those products with a chlorine content of 60% best resembled the pattern found in human milk. Consequently, technical PCB mixtures with a chlorine content of 60%, such as Aroclor 1260 and Clophen A60 were initially used as reference standards for quantification of PCB concentrations in human milk. For quantification, two or three characteristic peaks of the technical PCB mixture were related to the corresponding peaks in the human milk samples. Applying this approach, Acker and Schulte reported a mean PCB concentration of 3.5 ppm determined in 43 human milk samples on a lipid base. This mean concentration was similar to the mean level of 3.8 ppm (on a lipid base) reported for the sum of DDT and DDE (Acker and Schulte 1970a, b).

The determination of total PCB using a technical mixture as reference standard for calibration and quantification of PCB concentrations in humans does not take the metabolization of individual congeners into account resulting in markedly different compositions of individual PCB in the technical mixture compared to human milk. Results obtained with this technique varied widely between laboratories and were considerably influenced by the method of quantification chosen and by the technical PCB mixture used as a reference standard. Thus, results calculated as total PCB are prone to overestimation of the actual PCB concentration in human milk which must be considered when comparing recent results with historical analytical data generated in the 1970s based on determinations using packed GC columns. Chemical conversion methods, especially perchlorination, have also been used to determine total PCB concentrations in environmental and biological matrices. These methods are quite sensitive, but do not allow for peak pattern identification. Another drawback of perchlorination is that conversion of less chlorinated biphenyls is not quantitative (WHO 1993).

A breakthrough in the analysis of PCB in environmental and biological samples is attributed to the availability of capillary columns for gas chromatographic determination, which substantially enhanced the separation of complex mixtures (Schulte

and Acker 1974). In 1980, Ballschmiter and Zell investigated the composition of seven technical PCB mixtures by high-resolution thin-film glass capillary gas chromatography with electron-capture detection. Moreover, they developed a scheme of numbering the PCB congeners that follows the IUPAC rules of substituent characterization in biphenyls (Ballschmiter and Zell 1980). In 1983, Schulte and Malisch determined the contents of all individual PCB congeners in the two technical PCB mixtures Clophen A 30 and Clophen A 60 (Schulte and Malisch 1983).

The separation power provided by capillary columns allowed for the congener-specific analysis of the PCB pattern not only in technical PCB mixtures, but also in human milk. Moreover, it enabled an unequivocal determination of PCB congeners at trace concentrations due to separation from potentially overlapping lipophilic co-extracts. The PCB congeners with Ballschmiter and Zell numbers 138 (2,2′,3,4,4′,5-hexachlorobiphenyl), 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), and 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl) were identified as major contributors to total PCB contamination of human milk. A common approach to calculate the total PCB concentration in human milk based on the identified individual congeners was to multiply the concentrations of the PCB congeners 138, 153, and 180 by 7.03, 6.64, and 11.86, respectively. Summarizing these three products and dividing the sum by 3 yielded the total PCB content in human milk.

Schulte and Malisch showed that this approach leads to an overestimation of the actual PCB content in human milk. They reported that the sum of the dominating PCB congeners 138, 153, and 180 amounts to 55–70% (mean: 61%) of the PCB pattern in human milk. Based on this investigation, the authors proposed to calculate the "real" PCB content by multiplying the sum of the three PCB congeners by a factor of 1.64 (Schulte and Malisch 1984).

In 1984, Mullins et al. reported on the synthesis of all 209 PCB congeners, their spectroscopic properties, molar response factors, and retention times on a 50 m narrow bore fused silica capillary column (Mullins et al. 1984). Based on this investigation, Safe et al. reported on the first congener-specific analysis of the technical PCB mixture Aroclor 1260 and the PCB composition of a human milk extract (Safe et al. 1985).

Concentrations of PCB in human milk analysed in the 1970s/1980s are compiled in the WHO publication "Environmental Health Criteria 140" (WHO 1993). The data demonstrate the global distribution of PCB. In summary, it is stated that "the average concentrations of total PCB in human milk fat are in the range of 0.5-1.5 mg/kg fat, depending on the donor's residence, lifestyle, and the analytical methods used. Women who live in heavily industrialized, urban areas, or who consume a lot of fish, especially from heavily contaminated waters, may have higher PCB concentrations in their breast milk" (WHO 1993).

As mentioned above, the interpretation and comparison of these results, in particular for the evaluation of temporal trends is hampered by the different analytical approaches for the determination and quantification of the PCB concentrations in the human milk samples. A reliable temporal trend analysis can only be performed on a congener-specific basis. Reports on global surveys and/or spatial temporal trends of PCB comprising different time spans were repeatedly published (Schade

and Heinzow 1998; Solomon and Weiss 2002; Fürst 2006; Zietz et al. 2008; Ryan and Rawn 2014; Fång et al. 2015; Van den Berg et al. 2017; Brajenović et al. 2018). All these reports indicate a substantial decrease of PCB in human milk in countries where PCB have been banned or otherwise regulated. These publications often also contain a section related to the global contamination and temporal trend data on other POPs. The most comprehensive global data are compiled in the publication by Fång et al. (2015).

A subgroup of PCB consists of congeners that are not or mono-chlorinated at the ortho-positions of the PCB molecule. These compounds can adopt a co-planar structure and show toxic effects similar to specific polychlorinated dibenzo-*p*-dioxins and dibenzofurans and are thus denoted dioxin-like PCB (dl-PCB). Generally, their concentrations in human milk are substantially lower than those of the predominant PCB congeners. Due to the low levels and limited analytical sensitivity, a reliable analysis of dl-PCB in human milk was only possible by gas chromatography/high-resolution mass spectrometry from the mid-1990s onwards (see also Sect. 2.4).

2.3 Brominated Flame Retardants

2.3.1 Polybrominated Biphenyls

Due to the relatively easy non-invasive accessibility and its high lipid content, human milk was occasionally used as a matrix to explore the extent of human exposure to lipophilic persistent organic pollutants due to contamination incidents. One prominent example is the poisoning in Michigan 1973 (Fries 1985). In autumn 1973, the flame retardant Firemaster FF-1® was accidentally used instead of magnesium oxide in livestock feed and was fed to thousands of food producing animals resulting in severe adverse effects in these animals. Firemaster FF-1® consisted of Firemaster BP-6® and 2% calcium silicate as an anticaking agent. The flame retardant is a technical mixture of polybrominated biphenyls (PBB), predominately consisting of hexabromobiphenyls. Its main application was the use in polymers. The poisoning by Firemaster continued until April 1974 when the mix-up of feed additive and flame retardant was discovered and PBB were identified as the contamination source for the poisoning of the animals. The long contamination period led to high concentrations of these lipophilic compounds, in particular in cattle, chickens, and sheep. More than 30,000 cattle, 4500 swine, 1500 sheep, and 1.5 million chicken were killed (Fries 1985). Consumption of food derived from these animals resulted into a considerable human exposure. In an initial investigation in 1976 of 53 body tissue and 12 human milk samples from two areas in Michigan, concentrations were reported as 0.01–1.2 ppm, with a median of 0.068 ppm on a fat basis (Brilliant et al. 1978). In a larger investigation of 2986 human milk samples collected between May 1976 and December 1978 from all over Michigan, PBB were detected in 88% of the samples. The maximum concentration in milk was reported as 2.0 ppm, and the median and mean values were 0.06 and 0.1 ppm, respectively (Miller et al. 1984). In human milk fat from 32 directly exposed farmer's wives, the PBB concentrations were higher with a maximum of 92 ppm and a mean value of 3.6 ppm (Sonawane 1995).

Initially, the analysis of human milk samples was not performed as congener-specific but based on comparison and quantification based on technical mixtures on packed GC columns, similarly to the initial determination of PCB. In the middle of the 1980s application of capillary columns with mass spectrometric detection, in particular in negative chemical ionization mode (NCI) enabled the separation of technical PBB mixtures and revealed the congener-specific PBB pattern in human milk from various countries (WHO 1994a; Krüger 1988; Krüger and Groebel 1988). Most of the research was conducted with the Firemaster products as the PBB flame retardant with the highest production and application numbers. The predominant congeners in these products were found to be 2,2′4,4′,5,5′-hexabromobiphenyl (PBB-153) and 2,2′,3,4,4′,5,5′-heptabromobiphenyl (PBB-180) (WHO 1994a). Thus, the predominant PBB congeners carry the same substitution pattern as the major polychlorinated biphenyl congeners. This holds also true for the similar persistence of these compounds. Although banned since more than 40 years, especially PBB-153, the most bioaccumulative PBB congener can occasionally still be found in minor amounts in human milk samples collected in the 2000s (Fång et al. 2015).

2.3.2 Polybrominated Diphenylethers

Another class of brominated flame retardants (BFR) of particular importance are polybrominated diphenylethers (PBDE) which generally have replaced PBB after their phasing out. As additives to technical products, they can more easily leach out in contrast to reactive BFRs, which react with the protected product. In total, 209 individual PBDE congeners are possible. There are three technical mixtures PentaBDE, OctaBDE, and DecaBDE, termed according to their mean number of bromine atoms in the constituents of the commercial mixtures. Due to their high production and use since the late 1960s, PBDE have found global distribution (WHO 1994b). In the late 1990s, PBDE gained huge scientific and public interest when Meironyte et al. (1999) reported a time trend study concerning PBDE in Swedish human milk samples. The authors analysed archived pooled human milk samples which were collected at eight time periods between 1972 and 1997 for the PBDE congeners BDE-28, -47, -66, -85, -99, -100, -153, and -154. The sum of the concentrations of the eight PBDE congeners in human milk increased from 0.07 to 4.02 ng/g lipid during the 25-year period studied. While levels of other POPs in human milk decreased during this period, the PBDE showed a doubling of the concentration every 4–5 years. BDE-47, the predominant congener in the PentaBDE technical mixture, was found as the most abundant congener in all milk samples analysed. These results caused serious scientific and public concern and initiated worldwide comprehensive investigations on PBDE in human milk. The number of congeners studied varied widely. While almost all studies on human milk reported on the occurrence of BDE-47, -99, -100, and -153, the major constituents of the PentaBDE and OctaBDE technical mixtures, other studies covered a diverse range of further congeners. BDE-209, the predominant congener in the DecaBDE technical mixture was only occasionally analysed in the early human milk studies on PBDE. With increasing importance of DecaBDE as an alternative and replacement for

PentaBDE and OctaBDE technical mixtures, information on contamination of human milk by BDE-209 increased.

The variable number of PBDE congeners analysed and the reporting of total PBDE concentrations based on different congeners hampered the interpretation and comparison of results, especially in case of the early analysed human milk samples. This showed the need for a harmonized analysis of PBDE not only in human milk. Thus, in 2011, the Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) considered the following eight PBDE congeners to be of primary interest: BDE-28, -47, -99, -100, -153, -154, -183, and -209. The selection was based on the composition of the technical PBDE mixtures, occurrence in the environment, and available data on toxicity (EFSA 2011a). In 2014, the European Commission recommended that, besides other brominated flame retardants, further data on levels of these eight PBDE congeners in food and in humans should be gathered. Moreover, the European Commission recommended to also include BDE-49 and -138 into the analytical methods (EU Commission 2014). Therefore, the 10 above-mentioned congeners became more and more the standard set of analytes for the determination of PBDE in human milk.

Data on PBDE concentrations in human milk predominantly collected in European countries were compiled by EFSA in their risk assessments on PBDE in food (EFSA 2011a, 2023). Systematic reviews on global distribution and temporal trends of PBDE in human milk were performed by Lignell et al. (2009), Fång et al. (2015), Tang and Zhai (2017), Shi et al. (2018), Meng et al. (2021), and Gyllenhammar et al. (2021). The reviews showed that PBDE are globally found in human milk samples, however, with some distinct differences concerning their concentration. The contamination seems to be dependent on the application, type and extent of use, date of sample collection, and time point of legal restrictions for the different PBDE technical mixtures. In general, the reported levels, e.g., of BDE-47 were much higher in human milk samples from the USA compared to samples collected in other parts of the world (Fång et al. 2015), indicating the excessive use of the technical product PentaBDE in the USA due to certain flammability standards (Shaw et al. 2010; Charbonnet et al. 2020). The monitoring programmes also identified some hotspots, in particular in the vicinity of informal e-waste recycling where the PBDE contamination of human milk was substantially higher than in other areas with background contamination (Li et al. 2017).

Due to their persistence, bioaccumulation, and toxicological properties, the production and application of PBDE was widely banned or strictly regulated in the past 20 years and they were listed in the Stockholm Convention in 2009 (PentaBDE and OctaBDE) and 2017 (DecaBDE).

Lignell et al. (2009) and Gyllenhammar et al. (2021), in a follow-up study, investigated human milk samples collected between 1996 and 2017 from first-time mothers living in Uppsala/Sweden. The results showed decreasing levels of BDE-47, -99, and -100 during the study period. No significant time trend was found for BDE-153, however, a change point was observed around the year 2004 with increasing concentration before and decreasing levels after that year. This shift

in the PBDE profile is obviously caused by the subsequent substitution of the technical mixtures PentaBDE and OctaBDE.

While derivations of temporal trends are feasible on a national level provided that the human milk samples are collected from comparable cohorts, the deduction of global temporal trends is not meaningful due to the above-mentioned factors that have an impact on the analytical results. Concepts to derive reliable time trends from human milk studies of the WHO/UNEP-coordinated exposure studies are based on minimization of possible sources of variation from the sampling design and from chemical analysis (see Malisch et al. 2023, in Part I of this compendium, and the chapters on results and discussion in Part III, and specifically on time trends in Part IV of this compendium).

2.3.3 Hexabromocyclododecanes

Another group of brominated flame retardants that have been used widely as alternative to restricted PBDE are hexabromocyclododecanes (HBCDD[2]). Their technical mixtures consist of predominantly three isomers, denoted alpha-, beta-, and gamma-HBCDD of which gamma-HBCDD contributes most. As HBCDD isomers are lipophilic and persistent they could also be detected in human milk soon after their commercial introduction. Comprehensive overviews on occurrence of HBCDD in human milk were compiled by EFSA (2011b), Fång et al. (2015), Shi et al. (2018) and EFSA (2021). In contrast to the technical mixtures, where gamma-HBCDD dominates, the predominant isomer in human milk and other biological matrices is alpha-HBCDD which is the chemically most stable isomer and has the highest bioaccumulation potency (EFSA 2011b).

The analysis for HBCDD in human milk is either performed by GC-HRMS or by HPLC-MS/MS. The determination by GC-MS is mostly applied if HBCDD is analysed together with PBDE. However, this approach has the disadvantage that an isomer-specific determination is not possible as a separation of the three isomers cannot be achieved. Thus, the results represent the total HBCDD content. An isomer-specific determination of HBCDD which may be of importance for toxicological considerations is only feasible by application of HPLC-MS/MS. A direct comparison of HBCDD levels obtained by these two analytical approaches is hampered by the fact that the total HBCDD concentration determined by GC-HRMS is not necessarily equal to the sum of the three HBCDD isomer levels obtained isomer-specifically by HPLC-MS/MS analysis due to different response factors. This fact should be taken into account when data obtained with the two different analytical approaches are to be compared. This is especially important for the evaluation of time trends.

The above-mentioned publications indicated that the determination of a global time trend by consideration of occurrence data from different countries is not meaningful. This is due to the fact that the results are not only influenced by the variable characteristics of the participating cohorts but also depend on the HBCDD

[2] Sometimes in the literature also wrongly denoted as HBCD.

application date in the various countries, the length of use as well as on differences of entry into force of legal measures requiring prohibiting their further use.

The benefit of archived human milk samples was demonstrated for the determination of HBCDD concentrations by Fängström et al. (2008) who analysed human milk pools from Sweden which were archived between 1980 and 2004. The human milk pools showed a seven-fold increase of HBCDD concentrations between 1980 and 2002 with somewhat decreasing levels between 2002 and the end of the study in 2003/2004. Gyllenhammar et al. (2017) analysed several Swedish milk pools collected between 1996 and 2016 and reported a significant downward trend for the whole study period with a decrease of 2.0% per year. A significant change point was observed around the years 2002–2003 with an increasing trend before that year and a decreasing trend thereafter (Gyllenhammar et al. 2017).

2.4 Polychlorinated Dibenzo-*p*-Dioxins, Dibenzofurans and dl-PCB

Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are two classes of environmental chemicals that have no use and are not produced intentionally but are formed as unintentional and often unavoidable by-products in a number of combustion and technical processes. Due to their numerous sources, they have meanwhile found global distribution. Depending on the number of chlorine atoms and their structural position in the molecules, a total of 75 PCDD and 135 PCDF individual congeners can be differentiated. Both groups together are often termed "dioxins". The physico-chemical properties, sources, and basic information on toxic effects were compiled by the World Health Organization in 1989 (WHO 1989b).

While environmental specimens generally contain numerous PCDD/PCDF congeners, which often allow information on the sources, samples collected from mammals, including humans show only a reduced PCDD/PCDF profile predominantly consisting of congeners that are chlorine-substituted at the 2, 3, 7, and 8 positions. These congeners are of special importance as they are the most toxic ones and are only slowly metabolized. Due to their persistence and lipophilic properties, they are stored in fatty tissues and accumulate in the food chain. The most important route for human exposure to PCDD/PCDF is food consumption contributing over 90% of total exposure, with products of animal origin and fish making the greatest contribution to this exposure (Fürst et al. 1992; Liem and Rappe 2000).

As the PCDD/PCDF congeners do not exhibit the same toxic potency, toxicity equivalency factors (TEFs) were introduced that take into account the different toxicity in relation to the most toxic congener 2,3,7,8-TCDD. By multiplying the concentrations of the individual congeners in the analysed samples with the respective TEFs and summing up these values one gets the result expressed as toxic equivalent (TEQ) relative to 2,3,7,8-TCDD. Due to increasing knowledge on the toxicity of PCDD/PCDF, the TEFs were repeatedly re-evaluated and updated in the

past 40 years resulting in some substantial changes. The most frequently applied factors are I-TEFs (CCMS 1988), WHO_{1998}-TEFs (Van den Berg et al. 1998), and WHO_{2005}-TEFs[3] (Van den Berg et al. 2006). Currently, the WHO_{2005}-TEFs which are also the basis for legal provisions are almost exclusively used. The differences in the various TEF models have to be taken into account when comparing results, as the same analytical raw data after conversion to TEQ with different TEF models may substantially deviate. This hampers the interpretation and comparison of data reported as TEQ concentrations that were generated in the course of time by applying different TEF models.

Before the early 1980s, the analysis for PCDD/PCDF was generally limited to the determination of 2,3,7,8-TCDD following contamination incidents, such as in Seveso (Fanelli et al. 1982), or in Vietnam (Buckingham 1982). However, due to limited analytical sensitivity only relatively high concentrations could be measured.

The first measurement of 2,3,7,8-TCDD in human milk was reported by Baughman (1974), when he analysed 17 samples from the south of Vietnam collected in areas which were sprayed by the US army during the Vietnam war with Agent Orange, a mixture consisting of the phenoxy herbicides 2,4-D and 2,4,5-T which was highly contaminated with 2,3,7,8-TCDD. Concentrations of 2,3,7,8-TCDD in the human milk samples were reported to range between <1 (LOD) and 55 ppt[4] based on wet weight (Baughman 1974). Assuming a mean fat content in human milk of 3%, the 2,3,7,8-TCDD concentration in the sample with the highest contamination would amount to around 1830 ppt or pg/g lipid (Schecter et al. 1995).

In 1984, Rappe et al. were the first to report on the congener-specific determination of PCDD/PCDF in humans when they analysed five human milk samples from Germany (cited in WHO 1989b). Besides a concentration of 1.9 pg/g lipid for 2,3,7,8-TCDD, they also reported results for 14 further congeners with 2,3,7,8-chlorine substitution. While the highest level was found for octachlorodibenzo-*p*-dioxin (OCDD) at 434 pg/g lipid, the concentration of the other quantified congeners ranged between <1 and 72.8 pg/g lipid.

Until then, it was assumed that PCDD/PCDF, and in particular 2,3,7,8-TCDD can only be found in samples collected in areas following contamination incidents. As Germany was not considered as dioxin contaminated, the results were questioned but due to the great resonance in the public, great efforts were also initiated to evaluate the human milk results reported by Rappe et al. At the Dioxin Symposium 1985 in Bayreuth, Fürst et al. presented the results of the analysis of 50 human milk samples for PCDD/PCDF (Fürst et al. 1985, 1986). These results showed great similarities concerning congener profile and analyte concentrations with the data reported by Rappe et al. and thus confirmed that PCDD/PCDF cannot only be found in areas following specific contamination incidents but also in the background population. This was also confirmed by Ende (1986), Rappe et al. (1986), and Van den Berg

[3] As the re-evaluation of the WHO-TEFs took place in 2005, but the outcome was only published in 2006, the WHO_{2005}-TEFs are sometimes also denoted as WHO_{2006}-TEFs.

[4] Reported as ppt (parts per trillion), equal to pg/g or pg/mL.

et al. (1986) who performed first comprehensive surveys on PCDD/PCDF in human milk samples collected in different European countries. All samples showed a comparable congener profile almost exclusively consisting of PCDD/PCDF congeners with 2,3,7,8-chlorine substitution, i.e., the toxic congeners. Although the pattern showed great similarities, the concentrations of the individual samples varied to some extent.

Since the mid-1990s, the analysis of human milk for PCDD/PCDF was increasingly extended to the parallel determination of dioxin-like PCB (dl-PCB). Research on the toxicity of PCB has indicated that several congeners can adopt a co-planar structure and show toxic properties similar as certain PCDD/PCDF. The respective dl-PCB are either non-ortho or mono-ortho chlorine substituted. As a consequence, an expert group evaluated the different toxicities and proposed TEFs for a number of dl-PCB similar as for PCDD/PCDF (Ahlborg et al. 1994). These TEFs were re-evaluated by WHO in 1998 (Van den Berg et al. 1998) and 2005 (Van den Berg et al. 2006) and several changes were introduced. Due to their occurrence and toxic properties, the WHO in 1998 evaluated dl-PCB together with PCDD/PCDF in the derivation of a tolerable daily intake for the sum of these three contaminant classes (Van Leeuwen et al. 2000).

The contribution of dl-PCB-TEQ to total PCDD/PCDF/dl-PCB-TEQ in human milk differs depending on the TEF model applied. Generally, the PCDD/PCDF-TEQ is at least doubled if the dl-PCB-TEQ is added. In any case, a reliable interpretation and comparison of occurrence data expressed as TEQ results is only feasible if the same TEF model is used. Conversion of analytical raw data for PCDD/PCDF and dl-PCB in human milk with different TEF models can result in TEQ values that deviate by a factor of 2 or more.

Analyses of human milk, human blood, and adipose tissue have shown that levels of PCDD/PCDF and dl-PCB in human milk very well reflect the body burden as the concentration in these matrices is quite similar when expressed on a lipid basis (Todaka et al. 2010; Needham et al. 2011). This indicated that human milk can be a valuable matrix to estimate the human body burden with PCDD/PCDF and dl-PCB, and thus numerous studies were performed to assess the contamination of human milk with these contaminants at a global level. Comprehensive reviews on the contamination of human milk with PCDD/PCDF were published by Lakind et al. (2001), Lakind (2007), Srogi (2008), Ulaszewska et al. (2011), Fång et al. (2015), and Van den Berg et al. (2017). These reviews confirmed the global occurrence of PCDD/PCDF and dl-PCB in human milk and indicated that samples collected in industrialized areas generally show higher levels than samples collected in developing countries.

2.5 Chlorinated Paraffins

Although chlorinated paraffins (CP) have been produced since the 1930s, they only have attracted increased attention concerning human exposure in the past few years. CP are produced by chlorination of alkanes and consist of n-alkanes with varying

degrees of chlorination, usually between 40 and 70% by weight. According to their chain length, they can be divided into short-chain CP (SCCP) comprising 10–13 carbon atoms, medium-chain CP (MCCP) comprising 14–17 carbon atoms, and long-chain CP (LCCP) with 18 or more carbon atoms. CP with ≤9 carbon atoms are denoted very short CP (vSCCP). It is estimated that more than one million tons of CP are produced annually which China being the major producer. CP are used inter alia as high-temperature lubricants, plasticizers, and flame retardants in a wide variety of products. Technical CP are very complex mixtures which may consist of tens of thousands of congeners (Fiedler 2010; Van Mourik et al. 2016). CP may enter the environment during production, use, and improper disposal. As for other lipophilic persistent contaminants, food especially of animal origin is considered the main route of human exposure to CP.

The complexity of the technical CP mixtures impeded their analytical determination in environmental and biological samples in the past as no analytical method is able to separate the various constituents. A comprehensive overview on different analytical methods, their capabilities, limitations, and their area of application is given by EFSA (2020). A promising approach seems to be the combination of comprehensive two-dimensional gas chromatography (GCxGC) coupled with time-of-flight (TOF) mass spectrometry. Applying this technique, a separation into various homologue groups is feasible and also information on different chlorine substitution pattern can be received. The EU Reference Laboratory for halogenated POPs in Feed and Food developed a Guidance Document on the Analysis of Chlorinated Paraffins. This document compiles a set of analytical parameters that would lead to satisfactory method performance, provides an example of a method, and discusses different approaches of quantitative analysis of CP groups (chain length specific patterns) (EURL POPs 2021).

EFSA (2020) also compiled publications on CP concentrations in human milk from various countries. As these data were generated with different analytical approaches, the results should be compared with caution. In any case, the results show a broad range of contamination regarding CP in human milk, generally with higher levels of SCCP compared to MCCP. The results of Krätschmer et al. (2021) demonstrating that the CP levels analysed in human samples from various countries exceeded the PCB levels determined in the same samples should be considered as concern.

2.6 Per- and Polyfluoroalkyl Substances

Because of their high stability towards thermal, chemical, and biological degradation processes, as well as their inert and non-adhering surface properties, per- and polyfluoroalkyl substances (PFAS) have found a wide range of industrial applications. The group of PFAS comprises far more than 1000 known individual compounds. They are used in numerous commercial products, such as surfactants, lubricants, fire extinguishing foams, textile impregnation, electroplating, polishes, paintings, and many others (Glüge et al. 2020). PFAS consist of a hydrophobic alkyl

chain of varying length (typicallyC_4–C_{16}) and a hydrophilic end group, and thus exhibit amphiphilic properties. They may be released into the environment during production, use, and disposal, and have meanwhile found global distribution (Abunada et al. 2020). PFAS have been shown to be extremely persistent and some of them biomagnify.

First results on PFAS in human milk were only reported in 2004–2007. Kuklenyik et al. (2004) developed a high-throughput method for measuring trace levels of 13 PFAS (2 perfluorosulfonates, 8 perfluorocarboxylates, and 3 perfluorosulfonamides) in human serum and milk using an automated solid phase extraction (SPE) clean-up followed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). When analysing two human milk samples from the USA, they only found detectable concentrations for perfluoropentanoic acid (PFPeA 1.56 ng/mL) in one, and perfluorohexanoic acid (PFHxA 0.82 ng/mL) in the other milk sample, but no other PFAS in both samples.

So et al. (2006) analysed PFAS in human milk samples from 19 women from China. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were the two dominant compounds detected in all milk samples. Concentrations of PFOS and PFOA ranged from 0.05–0.36 ng/mL and 0.05–0.21 ng/mL, respectively. The maximum concentrations of other PFAS were 0.10 ng/mL for perfluorohexanesulfonate (PFHxS), 0.06 ng/mL for perfluorononanoic acid (PFNA), 0.015 ng/mL for perfluorodecanoic acid (PFDA), and 0.056 ng/mL for perfluoroundecanoic acid (PFUnDA). Similar results were reported for PFOS and PFOA in human milk samples from Japan by Nakata et al. (2007), and in human samples from Germany by Völkel et al. (2008).

Kärrman et al. (2007) studied occurrence levels of PFASs in human milk in relation to maternal serum together with the temporal trend in milk levels between 1996 and 2004 in Sweden. In this study, matched human milk and serum samples from 12 primiparous women in Sweden were analysed together with composite milk samples (25–90 women/year) collected from 1996 to 2004. PFOS and PFHxS were detected in all milk samples at mean concentrations of 0.201 ng/mL and 0.085 ng/mL, respectively. Perfluorooctanesulfonamide (PFOSA) was detected in eight milk samples with a mean concentration of 0.013 ng/mL, and PFNA was detected in two milk samples (0.020 and 0.014 ng/mL). PFOS and PFHxS levels in composite milk samples were relatively unchanged between 1996 and 2004, with a total variation of 20 and 32% coefficient of variation, respectively.

A key finding of the study by Kärrman et al. (2007) is the observation that the PFOS and PFHxS ratios between human serum and human milk are around 100:1 and 50:1, respectively. This indicates that in contrast to other POPs, human milk is not the matrix of choice in the evaluation of internal human body burden with PFAS because of the substantial lower concentrations in human milk compared to human serum.

2.7 Further POPs of Interest

The number of POPs analysed in human milk has constantly increased over the past 70 years. Following the identification of POPs in the environment, human milk samples were often analysed to monitor the extent of internal human exposure. Examples besides the already mentioned contaminants above which triggered comprehensive monitoring programmes, are e.g. **polychlorinated naphthalenes, pentachlorophenol, endosulfan, dicofol,** and emerging brominated flame retardants. Moreover, there are a number of chemicals that are proposed for further listing under the Stockholm Convention and thus may become part of human milk surveys, i.e., among others the pesticides methoxychlor and chlorpyrifos, and the UV absorber UV-328.

An overview of the Convention, the Global Monitoring Plan and its implementation by regional and global monitoring reports is given in Part I of this compendium (Šebková 2023). The regional and global monitoring reports as source of information for a wide range of POPs found in various environmental samples and human matrices should be pointed out.

So far, the analytical determination of POPs in human milk was generally performed by target analysis. This means that the method is optimized for the analyte of interest. Since a couple of years, the advances in analytical instrumentation in terms of sensitivity, resolution power, and data evaluation allow non-target analytical approaches, such as use of time-of-flight mass spectrometry (TOF-MS). Applying this technique, not only known POPs can be detected in human milk with high sensitivity, but also emerging contaminants which are hitherto unknown as contaminants in human milk (see also Šebková et al. 2023, in Part V).

3 First and Second Round of WHO Field Studies on Human Milk in the 1980s and 1990s

This section covers the early establishment of global harmonized human milk surveys studying impacts of exposure on human health. Because of the high toxicity of PCDD/PCDF, their occurrence, and levels observed in human milk in 1984 caused a considerable expert and policy debate and public concern about the safety of breastfeeding. Therefore, the WHO Regional Office for Europe in 1987 decided to carry out analytical studies on human milk, including interlaboratory quality control studies. The first results of the interlaboratory quality control study were already discussed during a WHO consultation in 1987, and the first part of the analytical field study was presented in early 1988. Eleven laboratories participated in the quality control study on PCDD/PCDF and six laboratories on PCB in human milk. In general, the results were in good agreement between the laboratories. The WHO Regional Office for Europe noted that interlaboratory quality control studies between laboratories performing the analyses of PCDD/PCDF, especially in human milk are vital to ensure the reliability and comparability of results.

The first part of the analytical field study comprised human milk samples collected between 1987 and 1988 in 19 countries. One of the main conclusions was that in general the levels of PCDD/PCDF in human milk tend to be highest in the most polluted and industrial areas. However, the differences were not significant as they were in about the same range as the analytical deviations found in the quality control studies. The results for the individual congeners analysed in human milk samples from the participating countries were compiled by WHO in 1989 (WHO 1989c). It was recommended that field studies on the determination of PCDD/PCDF and PCB should be repeated at 5 years intervals. Therefore, a second round of exposure studies was completed in 1992–1993. PCDD/PCDF and PCB were analysed in human milk samples from 47 areas in 19 countries. The data of the second field study which are reported on a congener-specific basis in detail showed that the levels of PCDD/PCDF were not increasing but tend to decrease in some participating countries (WHO 1996). The former recommendation to repeat field studies on human milk every 5 years was reconfirmed.

In addition, further quality control studies were completed in 1988–1989, and 1991–1992. Based on the results of the 1991–1992 quality control study, eight laboratories were accepted by WHO for the determination of PCDD/PCDF in human milk (WHO 1995).

In accordance with the recommendations from the third round of interlaboratory quality assessment studies, the WHO European Centre for Environment and Health (ECEH), Bilthoven Division, organized a fourth round of quality assessment studies on levels of PCB, PCDD, and PCDF in human milk and blood plasma in 1996/1997. The CVUA Freiburg, Germany, was the only laboratory that met all performance criteria for analyses of marker and dioxin-like PCB, PCDD, and PCDF in human milk. Consequently, this laboratory was designated as the WHO Reference Laboratory for the Third Round of the WHO-coordinated exposure studies (WHO 2000).

4 Lessons Learned from the Early Human Milk Surveys

Over the past 70 years, analysis of human milk has been demonstrated to be a good indicator for estimating human body burden in regions and on a global level. Benefits are the non-invasive sample collection and the relatively high lipid content which in connection with the modern instrumental capabilities allow an analytical identification and quantification of numerous POPs down to trace concentrations. These data can be used for estimation of exposure to the POPs for the breastfed infant.

There is presumably no other contaminant found in human milk that is as intensively researched as PCDD/PCDF. A number of surveys did not only focus on the concentrations of these POPs but also looked for parameters that have an impact on their levels in human milk. For this, comprehensive questionnaires were designed and send to the respective mother. These questionnaires usually ask for personal characteristics of the mother, such as age, weight, number of breastfed children, length of nursing period, food consumption, area of living, use of

cosmetics, smoking habits, and others. EFSA (2018) gives a comprehensive overview on these parameters and their impact on the concentrations in human milk. While the results were obtained for PCDD/PCDF, the outcome and conclusions can also be transferred to most of the other POPs found in human milk. In brief, the number of breastfed children and the length of the nursing period have a substantial impact. The higher the number of breastfed babies and the longer the breastfeeding period, the lower the concentration in the human milk. The special importance of food for POPs exposure was already demonstrated by Hayes et al. (1958) who concluded that there is strong evidence that fat of animal origin in the diet is the main source of exposure to DDT and DDE in subjects who are not occupationally exposed. This was subsequently also confirmed by other studies which showed that especially food of animal origin is the main route to human exposure with lipophilic persistent contaminants (Fürst et al. 1992; Liem and Rappe 2000). On the other hand, breastfeeding women who consume a vegetarian or vegan diet generally show somewhat lower POPs level in their breast milk. Due to the persistence of POPs, the levels in human milk are somewhat higher in mothers who breastfeed their first child at a higher age than respective younger first-time mothers (EFSA 2018). The area of domicile whether living in an urban or rural area has generally no impact on the POPs level in human milk provided that there is no hot spot in the vicinity. Finally, the impact of active and passive smoking of the mother on the contaminant levels is not clear as respective reports are inconclusive (EFSA 2018).

A comparison of results and an estimation of time trends based on data from different countries reported in the literature is severely limited due to the differences in sampling concepts (whether pooled or individual samples), and by the consideration or non-consideration of the above individual factors (diet, age, length of breastfeeding period, etc.) that may have a considerable impact on the contaminant concentration in human milk.

The information on potential impacts on the POP levels in human milk demonstrate the need for harmonized approaches for surveys that enable a reliable interpretation and comparison of results. Especially strict protocols for recruitment of participants with respect to age, number of breastfed babies, length of breastfeeding, and timepoint of sample collection during the breastfeeding period are required. Only if the participants of the surveys have a comparable core characteristic, the results of the analyses can reliably be compared. Further decisive survey criteria are distinct rules for sample collection, handling (volume, pooling), storage, and shipment of the samples to a laboratory as well as selection of a competent laboratory. Finally, validated analytical methods that are repeatedly and successfully tested in proficiency tests are a particular prerequisite for a sound performance of the human milk surveys.

Between 2000 and 2019, WHO and UNEP performed five rounds of global surveys on concentrations and trends of POPs in human milk, partly as joint studies (Malisch et al. 2023). After adoption of the Stockholm Convention, the number of POPs increased gradually not only to cover PCDD/PCDF, PCB, and organochlorine pesticides as "initial POPs" listed under the Stockholm Convention but also other

POPs, such as PBDE, HBCDD, polychlorinated naphthalenes, PFOS, PFOA, PFHxS, chlorinated paraffins, and others.[5]

The lessons from the previous WHO-coordinated exposure studies have also inspired the development and gradual updates of harmonized protocols for the WHO/UNEP-coordinated surveys focusing on identification of POPs.

The following chapters of this compendium describe the results of the global surveys on POPs in human milk from the Third Round of the WHO- and WHO/UNEP-coordinated exposure studies from 2000 onwards. As these surveys were based on strict protocols and requirements for performance of analysis by designated reference laboratories for chlorinated and brominated, respectively, fluorinated POPs, the data allow a reliable conclusion on global occurrence and potential time trends.

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Overview of WHO- and UNEP-Coordinated Human Milk Studies and Their Link to the Stockholm Convention on Persistent Organic Pollutants

Rainer Malisch, Karin Malisch, F. X. Rolaf van Leeuwen, Gerald Moy, Angelika Tritscher, Ana Witt, and Jacqueline Alvarez

Abstract

Building on the two rounds of human exposures studies coordinated by the World Health Organization (WHO) in the mid-1980s and 1990s to determine the concentrations of polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans in human milk, five further studies were performed between 2000 and 2019. Following the entering into force of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2004, WHO and the United Nations Environment Programme (UNEP) agreed to collaborate in joint studies. The collaboration aimed at supporting the Convention's

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_2

implementation by assessing its effectiveness as required under Article 16. It expanded the number of analytes in the studies to include the initial 12 POPs targeted by the Convention for elimination or reduction and subsequently to the 30 POPs covered under the Stockholm Convention as of 2019, furthermore two POPs proposed for listing.

The implementation of the studies has followed three basic steps: (1) collection of a large number of individual samples from mothers based on the standardized WHO/UNEP protocol; (2) from equal amounts of the individual samples, preparation of pooled samples that are considered to represent the average levels of POPs in human milk for a country or subpopulation of that country at the time of sampling; and (3) analysis of POPs in the pooled samples by the Reference Laboratories for the WHO/UNEP-coordinated exposure studies 2000–2019 (for chlorinated and brominated POPs in the period 2000–2019 at CVUA Freiburg, Germany, and for perfluoroalkane substances in the period 2009–2019 at Örebro University, Sweden).

In studies between 2000 and 2019, 82 countries from all United Nations regions participated, with 50 countries participating in more than one study. Repeated participation of countries permits the assessment of temporal trends, which can be used for risk management purposes as well as the evaluation of the effectiveness of the Convention in eliminating or reducing emissions of POPs.

Keywords

Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · Initial and new POPs · Global WHO/UNEP studies · Standardized protocol · Representative pooled samples · UN Regional Groups · Time trends

1 Introduction

For many years, human milk has served as a useful matrix to assess the overall exposure of the general population to persistent organic pollutants (POPs), such as DDT and other organochlorine pesticides (Schutz et al. 1998). The concentrations of most lipophilic POPs in human milk correlate well, in general, with those in maternal blood or adipose tissue. Therefore, monitoring the amounts of these chemicals in human milk, despite covering only women of childbearing age, has become an essential tool to determine the exposure of POPs in humans, including the exposure of breast-fed infants, who have higher intakes on a bodyweight basis than adults (Fürst 2023, in Part I of this compendium).

In the mid-1980s and early 1990s, the World Health Organization (WHO) coordinated two exposure studies on concentrations of polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) in human milk (WHO 1989, 1996). After the adoption of the Stockholm Convention on Persistent Organic Pollutants (POPs), hereinafter the Convention, in 2001 (UNEP 2001), WHO and the United Nations Environment

Programme (UNEP) agreed to collaborate in joint studies starting in 2004 to support the implementation of the Convention by assessing its effectiveness as required under its Article 16. Between 2000 and 2019, WHO and the United Nations Environment Programme (UNEP) performed five global studies on concentrations of POPs in human milk with the participation of 82 countries, which included the assessment of time trends.

Human milk is a core matrix under the Convention's Global Monitoring Plan (GMP) for POPs. The objective of human biomonitoring within the GMP, which includes the WHO- and UNEP-coordinated human milk studies, is to identify temporal and, as appropriate, spatial trends in levels of POPs in humans to evaluate the effectiveness of the Convention.

Between 2000 and 2019, the scope of POPs increased from the three POPs of interest in the first WHO-coordinated studies in a first step covering the initial 12 POPs listed in Annexes A (for elimination), B (for restriction), or C (for reduction of releases from unintentional production) of the Convention at its adoption in 2001. As of 2019, the list of analytes in the human milk studies includes 30 chemicals and reflects all the amendments by the Convention's Conference of the Parties (COP) to date. Many of these chemicals have numerous congeners, homologous groups, isomeric forms, and transformation products, which significantly increases the number of recommended analytes (see Table 1 in Sect. 4). An overview of the Stockholm Convention, the Global Monitoring Plan and its implementation by regional and global monitoring reports is given in Part I of this compendium (Šebková 2023).

2 Development of WHO/UNEP-Coordinated Exposure Studies Over Time

2.1 WHO Exposure Studies 1987–1988 (First Round) and 1992–1993 (Second Round)

In the mid-1980s, the WHO's Regional Office for Europe (WHO/EURO) initiated a comprehensive program to assess the possible health risks of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF). This program was carried out in collaboration with other international organizations and national institutions. It concentrated particularly on the health risk of infants due to exposure through contaminated human milk and aimed at preventing and controlling exposure to these environmental chemicals. Because of the fat content (about 4%), human milk represents a convenient matrix easy to collect in a non-invasive manner matrix to estimate the levels of PCB, PCDD, and PCDF that are comparable to levels in plasma, serum, and adipose tissue, when calculated on lipid basis. Levels of these contaminants in human milk are indicators of the cumulative exposure of women at the age of their first pregnancy.

The first WHO/EURO-coordinated exposure study on concentrations of PCB, PCDD, and PCDF in human milk took place in 1987–1988 with participants from 12 European countries and seven countries outside Europe reporting results for PCDD and PCDF. In addition, eight European and three non-European countries submitted results for the so-called marker PCB, namely, PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180 (WHO 1989).

In the second round in 1992–1993, a total of 19 countries (17 European, Canada and Pakistan) participated for determinations of PCB, PCDD, and PCDF, which included marker PCB and dioxin-like PCB. Pooled samples from areas within countries having different environmental pollution conditions were analyzed (WHO 1996). Interlaboratory quality control studies were performed to ensure reliability and comparability of the results from the exposure studies (WHO 1989, 1991). Results of these two rounds were reviewed by Fürst 2023.

2.2 WHO Exposure Study 2000–2003 (Third Round)

The third round of the WHO-coordinated exposure studies started in 2000 with the aim of (1) producing reliable and comparable data on levels of PCB, PCDD, and PCDF in human milk to further improve the health risk assessment for infants; (2) determining time trends in exposure levels in the countries and areas already studied during the first and second rounds between 1986–1988 and 1992–1993, respectively, and (3) providing an overview of exposure levels in various countries and geographical areas. In addition to the collection of human milk samples from women, who were exposed through the consumption of contaminated foods up until the birth of their first child, the study protocol also provided the option to include samples from possibly highly exposed local populations to provide dietary intake guidance for risk management purposes. To collect data from more countries, including those outside the European region, this study was organized in collaboration with the International Programme on Chemical Safety (IPCS) hosted at WHO and the WHO Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS Food).

To ensure the reliability of exposure data and improve comparability of analytical results among different laboratories during the third round of global human milk studies, WHO implemented a quality assessment study on the determination of PCB, PCDD, and PCDF levels in human milk samples. The goal was to identify laboratories, whose results could be accepted by WHO for exposure assessment purposes. The WHO report presents the results of the study, including a list of qualified laboratories for each of the studied compounds (WHO 2000a; for conclusion, see Sect. 3.7 in the following).

The third round was performed from 2000 to 2003 with the determination of PCB, PCDD, and PCDF in samples from 26 countries/regions (Malisch and van Leeuwen 2003).

2.3 Stockholm Convention on Persistent Organic Pollutants: Expansion of Objectives and Analytes

The Stockholm Convention on Persistent Organic Pollutants was adopted in 2001 and entered into force in May 2004. The objective of the Convention is to protect human health and the environment from certain POPs by reducing or eliminating their production and releases (UNEP 2001; Šebková 2023).

POPs are a group of organic chemicals that have been intentionally or inadvertently produced and introduced/released into the environment. Due to their stability and lipophilic properties, they (1) remain intact for exceptionally long periods of time (many years), (2) become widely distributed throughout the environment, (3) accumulate in the living organisms, bioaccumulate in the food chain and therefore, are present in the human body mainly in adipose tissue and expressed in human milk during lactation; and (4) are toxic to both humans and wildlife.

An assessment report on 12 selected POPs had been prepared by the International Programme on Chemical Safety for preparation of an international legally binding instrument for implementing international action on certain POPs and presented at the first session of an International Negotiating Committee in 1998 (Ritter et al. 1998). These 12 chemicals or groups of chemicals were included by the Convention initially—organochlorine pesticides, such as aldrin, chlordane, and DDT, industrial chemicals, such as PCB, and unintentionally generated chemicals, such as PCDD and PCDF. Until 2019, another 18 chemicals or groups of chemicals have been covered by the Convention as POPs.

Article 16 of the Convention requires periodic effectiveness evaluations. To facilitate such evaluation, the Conference of the Parties established arrangements to provide itself with comparable monitoring data on the presence of the chemicals listed in Annexes A, B, and C as well as their regional and global environmental transport. They include reports to the Conference of the Parties on the results of the monitoring activities on a regional and global basis at intervals specified by the Conference of the Parties. Reports and other monitoring information are significant elements of the evaluation (UNEP 2001; Šebková 2023). The first six-year evaluation cycle took place between 2010 and 2017 (UNEP 2017a).

In 2003, UNEP anticipating the Article 16 requirement convened a workshop to consider modalities for providing comparable and reliable monitoring data on the POPs included in the Convention. The workshop identified human milk as a preferred matrix for monitoring and specifically recognized the existing WHO program with established detailed protocols. In particular, the WHO studies fulfilled important requirements for biomonitoring (UNEP 2003).

2.4 Pilot Study

With the expansion of the number of contaminants under the Stockholm Convention, a pilot study was conducted in 2003 by WHO and CVUA Freiburg as the Reference Laboratory for the third round of WHO-coordinated exposure studies with

fat extracted from the human milk samples retained from the 2000–2003 round with the aim of assessing the feasibility of measuring the initial 12 POPs listed by the Convention in human milk. This capability of measuring all 12 POPs in pooled human milk samples was confirmed with the introduction of additional analytical steps. Summarizing results of this pilot study together with the results of the following fourth round (2005–2007) were presented on regional basis (Malisch et al. 2008). The pilot study also included selected polybrominated diphenyl ethers (PBDE) (Kotz et al. 2005), which were candidates for future inclusion in the Convention at that time, and alpha-HCH, beta-HCH, gamma-HCH and endosulfan, which were added to the Convention later.

2.5 Joint WHO/UNEP Study 2005–2007 (Fourth Round)

In 2005, WHO and UNEP agreed in a Letter of Understanding that WHO human milk studies should be performed in close collaboration with UNEP, which acted as the interim secretariat of the Stockholm Convention. The results should be used to identify possible global temporal trends of concentrations of POPs in human milk and to assess whether the Convention is serving as an effective tool to eliminate or reduce emissions and reduce human exposure to these POPs.

With the new scope and as the most cost-effective approach, the previous WHO protocol for the collection, handling, and analysis of human milk samples, which was limited to PCB, PCDD, and PCDF only, was considerably modified in order to more accurately assess changes in concentrations of POPs over time based on statistical considerations on the required number of individual samples for preparation of the pooled samples (WHO 2005; see Sect. 3). Based on the results of the pilot study (see Sect. 2.4 above), the fourth global exposure study was jointly performed in 2005–2007 by WHO and UNEP (Malisch and Moy 2006; Malisch et al. 2008) with pooled human milk samples from 13 countries for the 12 POPs analyzed by CVUA Freiburg serving as the Reference Laboratory. It should be noted that one sample that was submitted in 2004 by a country was counted in this round as well. The study already included nine of the 11 candidate POPs, which were then listed in 2009, 2011, and 2013 (for the expansion of analytes of interest over time, see Sect. 4; for results, see UNEP 2013a).

2.6 Joint WHO/UNEP Study 2008–2012 (Fifth Round)

In 2007, the Conference of the Parties (COP) to the Stockholm Convention adopted the Global Monitoring Plan (GMP) for POPs. Monitoring of human milk and blood serum, as indicators for human exposure, and ambient air were identified as key elements for evaluating the effectiveness of the Convention. These sample materials are considered core media for POPs monitoring in the frame of the GMP for POPs (UNEP 2007a).

A guidance document for the GMP was developed with more detailed recommendations for the monitoring of these core matrices (UNEP 2007b) and is continuously updated to address the listing of new POPs, the most recent version being issued in 2019 (UNEP 2019a).

The fifth round of human milk monitoring was performed as a joint WHO/UNEP-coordinated exposure study between 2008 and 2012 following a revised protocol (WHO 2007) with two elements:

- The first part initiated by WHO GEMS/Food and supported by the Global Environment Facility (GEF) was performed between 2008 and 2010.
- The second part was initiated by UNEP that was supported by GEF (UNEP/GEF POPs GMP projects) and by the Strategic Approach for International Chemicals Management's Quick Start Programme (SAICM QSP) to include countries in regions that were under-represented in earlier studies. From 2009 to 2011, UNEP/GEF POPs GMP projects were implemented in 31 countries in the Pacific Islands, Africa (West Africa and South-East Africa), and Latin America. During the same period, two SAICM QSP projects were implemented in four Caribbean island countries.

For consistency in its measurements and its comprehensive quality control program, CVUA Freiburg analyzed all of samples. In order to provide baseline data for the effectiveness evaluation of the Convention for newly included POPs, UNEP requested CVUA Freiburg to broaden the analytical scope of the study to include all POPs added to the Convention in 2009 as well as candidate POPs under review in 2011 (see Sect. 4). Perfluorooctane sulfonic acid (PFOS) and related compounds, which were added in 2009 to the list of chemicals for restricted use, were likewise analyzed at the Örebro University, Sweden (see Sect. 3.7).

An interim status report on the jointly conducted human milk survey with results of 7 from 25 countries and temporal trends for PCDD/PCDF in 17 countries was provided to the fourth COP in 2009 (UNEP/WHO 2009). Results for PCDD, PCDF, PCB, DDT, and hexachlorobenzene (HCB) for 23 countries (from both parts of the fifth round) were presented at the International Symposium on Halogenated Persistent Organic Pollutants in 2010 (Malisch et al. 2010) and for additional POPs in 2011 (Malisch et al. 2011). A global overview of results of the 2005–2007 and 2008–2010 samples was submitted to the fifth COP in 2011 (UNEP 2011a). Results for concentrations for the initial 12 POPs from 30 pooled samples from countries from Africa, Latin America, and the Pacific Islands participating in the second part (2009–2011) were summarized separately (UNEP 2013b; Fiedler et al. 2013). Overall, 49 countries participated in both parts of the fifth round from 2008 to 2012.

A comprehensive report for the sixth COP in 2013 provided an overview on all samples of the third, fourth, and fifth rounds, spanning the period 2000–2012. It revealed large global differences among various POPs and a decreasing trend in PCDD and PCDF levels in a number of countries (UNEP 2013a). Also, aspects of the risks and benefits of breastfeeding were discussed and later published in more detail (van den Berg et al. 2016).

2.7 UNEP-Studies 2014–2015 (Sixth Round) and 2016–2019 (Seventh Round)

At its sixth meeting in 2013, the COP adopted a decision on the GMP for the effectiveness evaluation, which welcomed the compilation of the results of the first phase of the global human milk studies with data for the period 2000–2012 (UNEP 2013a) and encouraged parties to participate in second-phase milk studies to enable a harmonized determination of global and regional trends in human exposure to POPs (UNEP 2013c). Therefore, UNEP initiated the sixth round of WHO/UNEP human milk studies performed in 2014–2015. The objective was to generate a second phase of human milk data specifically for the effectiveness evaluation of the Stockholm Convention as required by Article 16. Samples from 13 countries were analyzed for this round. It should be noted that 12 samples were collected during 2014 and 2015, whereas one country submitted its sample in 2013, which is included in this study as well. Data were produced on all 23 POPs listed in the Convention as of 2013. They provided an indication of changes in concentrations over time and were used as a contribution to the required evaluation of effectiveness in protecting human health and the environment from POPs.

As a continuation, a comprehensive study of human milk samples was supported by GEF and performed during 2016–2019, which received samples from 36 countries from Africa, Asia, Latin America, and the Pacific Islands. Furthermore, seven European countries participated. A revised version of the original protocol for collecting of the samples was used (UNEP 2017b). Initially, monitoring of 23 POPs listed by the COP in 2013 was required. Later, in agreement with UNEP, the Reference Laboratories expanded the analytical spectrum with the inclusion of the seven POPs that were listed in 2015 and 2017 as well as possible candidates for the COP in 2019. Therefore, for the seventh round (2016–2019) results for all 30 chemicals that were listed up until 2019 are available.

2.8 Self-Funded Countries

Throughout the different rounds, many countries participated in the studies without support from GEF or other donors. These countries submitted samples based on internal priorities and resources throughout the whole period 2000–2019.

3 Concepts and Protocols

Generally, the concept of the WHO/UNEP-coordinated exposure studies has four basic elements:

1. Collection of individual samples from mothers fulfilling protocol criteria
2. From equal aliquots of individual samples, preparation of pooled (physically averaged) samples that are considered to represent the average levels of POPs

in human milk for a country or a subpopulation of that country at the time of sampling
3. Analysis of these pooled samples in Reference Laboratories to ensure the reliability of the exposure data and to improve the comparability of analytical results
4. Repeated participation of countries allowing conclusions on temporal trends

3.1 Protocols

To ascertain comparability of results, human milk samples were collected following WHO- and UNEP-designed protocols during the studies performed between 2000 and 2019 under the supervision of a National Coordinator in each country. The protocols deal primarily with the number and type of the individual samples, selection of donors and procedures for collecting, storing, pooling, and shipping of samples to the Reference Laboratory for analysis.

Ethical aspects were addressed as the initial protocol underwent an evaluation by the WHO Research Ethics Review Committee. The requirements of national ethics committees were also met, including informed consent. The identities of all donors were kept confidential by the National Coordinator. Furthermore, the clear and consistent communication of the health benefits of breastfeeding for both the mother and infant was an important element.

The guidelines for collecting of the samples were intended to assist the National Coordinator in each country in developing a national protocol. The protocols differ in details both as a result of changes in the WHO- and UNEP-designed protocols and requirements at the national level. The following protocol versions of the WHO- and UNEP-coordinated exposure studies were used:

- Third round (2000–2003) and the sample of 2004 (WHO 2000b)
- Fourth round (2005–2007) (WHO 2005)
- Fifth round (2008–2012) (WHO 2007)
- Sixth round (2013–2015) (UNEP 2012)
- Seventh round (2016–2019) (UNEP 2017b)

3.2 Collection of Individual Samples

All guidelines for collecting the samples are based on the following general principles in conducting studies involving donors of human milk:

- Breastfeeding in all instances should be promoted and supported
- A sampling of milk should neither be an undue burden to the mother nor compromise the nutritional status of the infant

For the comparison of population-based results, random donor selection is critical for obtaining reliable and comparable data. In order to detect small changes in levels

of POPs, variability has to be limited as far as possible while maintaining a reasonable pool of qualified donors. The criteria for the selection of donors were designed to reduce factors that are known to influence the levels of POPs in human milk. Being a *primipara* (giving birth for the first time) is the most important criterion as these levels are known to decrease during breastfeeding (Lakind and Berlin 2002). As explained above, the protocols differ in details. For the selection of donating mothers for the third round (2000–2003), the following criteria were applied:

- donors: *primiparae*
- mother and child apparently healthy and pregnancy normal
- exclusively breastfeeding
- one child (i.e., no twins) and
- residing in an area for about 5 years

With minor modifications, e.g. the additional requirement of the 2004 protocol that mothers should be under 30 years of age, the criteria listed above were applicable in all exposure studies. Questionnaires for potential human milk donors should be completed well before delivery to help select potential donors as early as possible. However, in practice, this was not always possible. If necessary to assure a sufficient number of donors, the National Coordinator could waive certain requirements, such as the age limitation.

3.3 Number of Individual Samples and Representative Pooled Samples

In order to get statistically reliable data, an appropriate number of qualified donors should be identified prior to providing samples. The third round (2000–2003) was based on pooling of 10 individual samples. Breast milk from well-defined groups of mothers living in at least two areas with different exposures was to be collected and pooled—for example, one from an exposure group expected to be high and another from a representative exposure group, with preferably additional pooled samples if possible. Most countries in the third round submitted two or three pooled samples, while 13 pooled samples were received from the Hong Kong Special Administrative Region (SAR) of China reflecting different dietary intake groups.

For the effectiveness evaluation of the Stockholm Convention, temporal trends in levels of POPs in human milk need to be assessed. For this purpose, the determination of small changes in levels of POPs is necessary and requires that variability and uncertainty in the sampling process be limited as far as possible, while maintaining an adequate number of qualified donors. Therefore, the revised WHO protocol guidelines for the fourth study (2004–2007) and subsequent rounds called for the recruitment of 50 individual donors per pooled sample in countries with up to 50 million population instead of 10 as previously recommended. A report entitled "Simulation of statistical analyses" provides the statistical considerations of this

revised sampling (WHO 2007). Starting in 2005, the option to include pooled samples from possible high-exposure groups was discontinued (WHO 2005).

It is recognized that some flexibility is necessary for countries with small populations and/or low birth rates. In some cases, reducing the number of donors was unavoidable. In general, countries with populations greater than 50 million are asked to add at least one additional participant per one million population over 50 million. Countries with populations well over 50 million are encouraged to prepare a second pooled sample (or more) if feasible.

3.4 Preparation of Individual and Pooled Samples

In order to minimize contamination of the human milk samples, the protocols advised purchasing glass bottles and procedures for their cleaning. Glass was chosen as the most suitable material despite certain disadvantages, such as heavier weight, breakage potential, and possible presence of fluorine-containing plastic caps. For each participant of UNEP/GEF projects, the Reference Laboratory for chlorinated and brominated POPs (CVUA Freiburg) purchased, cleaned, and shipped glass bottles to these countries, as described in the protocol (UNEP 2017b).

Since 2005, the protocol specified the collection of 50 individual samples of 50 ml each. Prior to subdividing these, the samples were homogenized by shaking for 10 min. For the analysis of analytically simple POPs, such as old pesticide POPs and marker PCB, a 25 ml aliquot of each individual sample was taken and sent to a qualified laboratory chosen by the National Coordinator. For the pooled sample, the remaining 25 ml from each of the 50 individual samples were used to make one pooled sample of 1.25 l and shipped frozen to CVUA Freiburg as the WHO/UNEP Reference Laboratory for chlorinated and brominated POPs in human milk for the 2000–2019 surveys.

This approach, which involves the national analysis of basic POPs, was also intended to foster national quality control studies and support capacity building in participating countries: The comparison of the mean of the individual samples for these analytes with the result of the Reference Laboratory serves as an internal check, as the average of the results of the individual samples should be comparable to the result of the pooled sample, which is prepared from equal aliquots of the individual samples. For other aspects regarding individual and pooled samples, see Sect. 3.6 in the following.

For analysis of PFOS and related substances, CVUA Freiburg sent a 10 ml aliquot of the pooled sample to Örebro University serving since 2009 as the reference laboratory for this project for these compounds. After analysis, any remaining pooled sample was stored at CVUA Freiburg in the Global Human Milk Bank at −20 °C. The bank is used when new POPs are added to the Stockholm Convention to allow for a retro-perspective analysis. Figure 1 illustrates the flow of samples.

In some cases, when frozen receipt by the reference laboratory could not be guaranteed, a small amount of potassium dichromate ($K_2Cr_2O_7$) was added to the

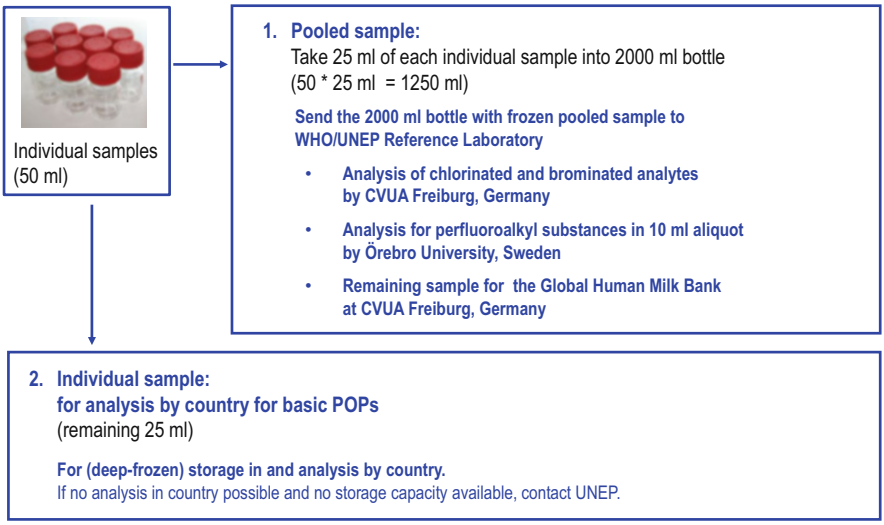

Fig. 1 Concept for individual and pooled samples

sample for stabilization (resulting concentration in human milk about 0.1% w/w) (Malisch 2001; Schecter et al. 2004; UNEP 2012).

3.5 Cost-Effectiveness of Analysis of Pooled Samples

In all WHO- and UNEP-coordinated exposure studies of human milk, only pooled samples were used for analysis. By analysis of one or a few pooled human milk samples considered to represent a country, an estimate of the average human body burden can be obtained, which is the result of long-term exposure in different countries of the world. The analysis of one or a few pooled human milk samples considered to be representative is far less expensive than the analysis of a high number of individual samples, particularly for PCDD and PCDF.

Altogether, 232 pooled samples were submitted between 2000 and 2019 (see Sect. 6.2). This number of pooled samples considered to represent a country provided the same information as would be received by calculation of the mean of more than 2000 individual samples (assuming 10 individual samples per pool) or more than 11,000 individual samples (assuming 50 individual samples per pool). Alternatively, human exposure in a country could be estimated by analyzing hundreds of different foods reflecting the main sources of exposure. However, this would be extremely costly and fraught with a significant number of other variabilities and uncertainties influencing exposure, such as food consumption and body weights of population subgroups. In conclusion, an important advantage of the analysis of pooled human milk samples is its cost-effectiveness. Furthermore, the relative uncertainty is reduced by the physical averaging procedure because equal

aliquots of many individual milk samples are combined to form a national pooled sample.

3.6 Variation of Individual Samples

As noted above, analyzing pooled samples considered as representative is a highly efficient way to get a general overview of mean levels of various POPs in a country. On the other hand, the analysis of individual samples (from individual donors) can provide information on the distribution of exposures and on factors possibly contributing to exposure. Individual samples can span a broad range of concentrations. If significantly elevated levels are found in pooled samples, a follow-up is usually recommended; if levels are quite low, no particular additional effort would seem to be necessary. This allows the saving of time and resources.

Since 2005, the WHO and UNEP guidelines recommend the analysis of the aliquots of the individual samples in laboratories in that country where capacity exists for legacy POPs, e.g., the organochlorine pesticides DDT, dieldrin and endrin, and indicator PCB in laboratories in that country. These compounds can be determined by analytical methods using basic instrumentation (gas chromatography with specific detectors) that are available in many developing countries.

3.7 Reference Laboratories

The analytical performance of a laboratory contributes to both the accuracy and precision of results and, therefore, can enhance the interpretation of time trends. To ensure the reliability of exposure data and improve comparability of analytical results among different laboratories, WHO had coordinated a number of interlaboratory quality assessment studies. The fourth quality assessment study on levels of PCB, PCDD, and PCDF in human milk was conducted with the objective of identifying laboratories whose results could be accepted by WHO for the third round of exposure assessments. The final report presents the results of the study, including a list of qualified laboratories for each of the studied compounds (WHO 2000a). The CVUA Freiburg, Germany, was the only laboratory that met all performance criteria for analyses of marker and dioxin-like PCB, PCDD, and PCDF in human milk. Consequently, this laboratory was designated as the WHO Reference Laboratory for the Third Round of the WHO-coordinated exposure studies and studies thereafter.

The successful performance of the pilot study for the expansion of analytes provided the opportunity to expand the scope of the studies by the inclusion of all 12 initial POPs (see Sect. 2.4 above). To further ensure consistency in measurements of the subsequent exposure studies organized by WHO and UNEP, all samples were analyzed for the chlorinated and brominated POPs listed in the Stockholm Convention by CVUA Freiburg as the Reference Laboratory using validated methods (UNEP 2013a, 2017b). The annual successful participation in international

proficiency tests has been part of the comprehensive quality control program of CVUA Freiburg as an accredited laboratory during the period 2000–2019.

In 2006, CVUA Freiburg was designated as the European Union Reference Laboratory (EU-RL) for PCDD, PCDF, and PCB in feed and food and as the EU-RL for pesticide residues in food of animal origin and commodities with high fat content (European Commission 2006). In 2018, the tasks of the EU-RL for PCDD, PCDF, and PCB in feed and food were extended to all halogenated POPs (European Commission 2018). For the analysis of human milk for WHO- and UNEP-coordinated exposure studies, complementary responsibilities of CVUA Freiburg had significant synergistic effects, in particular the development of analytical methods and quality control.

With the inclusion of PFOS and related compounds in 2009, additional expertise was needed and perfluorinated chemicals were analyzed at the Man-Technology-Environment (MTM) Research Centre of Örebro University, Örebro, Sweden (UNEP 2013a, 2017b).

For all samples of the WHO/UNEP-coordinated exposure studies, rigid quality control programs were carried out by the reference laboratories to ensure high quality of data and comparability of results (for chlorinated and brominated substances, see quality control data in the respective analytical chapters of Part II of this special issue).

As explained above in Sect. 3.4, the analysis of pooled samples by the WHO/UNEP Reference Laboratory and the option to have the individual samples analyzed for old pesticide POPs and marker PCB in a competent national laboratory is a contribution to capacity building, particularly in developing countries.

By performing the analysis of pooled samples (considered to be representative for the participating countries) at the Reference Laboratories, a high degree of reliability of the analytical results can be achieved. Such data are essential to statistically validate changes in concentrations of POPs over time in accordance with the guidance of the GMP for POPs (UNEP 2019a), as assessed specifically for PCB, PCDD, and PCDF (Malisch et al. 2023a, in Part IV of this compendium), DDT, beta-HCH, and HCB (Malisch et al. 2023b, also in Part IV), PBDE (Schächtele et al. 2023, in Part III), and PFAS (Malisch et al. 2023c, in Part IV).

3.8 Biosafety

One of the criteria for selecting potential donors is that both the mother and infant should be apparently healthy and that mothers had a normal pregnancy. Possible health risks to staff handling the samples can be caused by infections, such as infectious hepatitis or AIDS when the donors are not aware or do not inform about the infection otherwise screened during pregnancy and excluded. However, while the infectivity of human milk from possibly HIV-positive mothers is considered to be low when ingested by infants, appropriate precautionary measures should be taken if the health status of the donor is questionable. In such situations, milk should be considered infectious until it is decontaminated. In this regard, any milk sample

known or suspected to be contaminated with HIV should be decontaminated by heating at about 60–65 °C for 30 min. This is particularly important for countries with high HIV prevalence and limited HIV screening.

4 Analytes of Interest: Expansion Over Time

When the WHO-coordinated exposure studies started in the mid-1980s, they initially focused on PCB, PCDD, and PCDF. With this focus, the third round was started in 2000. With the ratification of the Stockholm Convention, UNEP started its collaboration with WHO to expand the spectrum of analytes to cover all 12 POPs listed in the Convention's annexes.

The number of analytes in the global human milk studies has been continuously expanding as new POPs were listed in the Annexes A (for elimination), B (for restriction), and C (for reduction of releases from unintentional production) of the Convention. At the fourth COP in 2009, nine new POPs were added (UNEP 2009). Altogether, nine additional POPs were listed at the fifth through the ninth COPs held from 2011 to 2019 (UNEP 2011b, 2013c, 2015, 2017c, 2019b) increasing the number of listed POPs to 30 chemicals or groups of chemicals (28 chlorinated or brominated, 2 perfluorinated) (UNEP 2020). Many of these chemicals have numerous congeners, homologous groups, isomeric forms, and transformation products, which significantly extends the number of recommended analytes (UNEP 2019a). Furthermore, two chemicals proposed for listing under the Convention were of interest. These 30 listed POPs along with their related products of toxicological concern and the two POPs of interest proposed for listing under the Convention are shown in Table 1.

This expansion spanned a period of about 20 years. Depending on the time of submission of the samples and availability of sufficient sample volumes, previous samples were retrospectively analyzed for new POPs. Following this approach, it was possible to obtain information on the presence of candidate POPs even before they have been listed. The last round (2016–2019) started with 23 POPs (as listed until the sixth COP in 2013) to be monitored. In 2018, it was decided to also determine concentrations of the other POPs, which were listed at the seventh COP in 2015 and eighth COP in 2017, and two possible candidates for the ninth COP in 2019. Thus, samples taken during the most recent survey (2016–2019) were analyzed for all 30 POPs listed and in addition for two possible candidate POPs. It should be noted that for small countries, the collection of the desired number of individual samples for a certain round was not always possible. In some cases, the collection of the recommended sample volume, i.e., 50 ml from the individual mothers, was not possible. Therefore, in few cases, a smaller subset of POPs was analyzed. However, data on all 30 listed POPs and two additional POPs proposed for listing are available for most human milk samples of the 2016–2019 period.

Table 1 Chemicals and analytes recommended for analysis of the core matrix "human milk" (as of 2019, 30 listed POPs and two chemicals of interest proposed for listing)

	COP No.	Year	Parameter	Annex	Parent POPs	Transformation products
		2001	**1. Initial 12 POPs**			
1			Aldrin	A	Aldrin	
2			Chlordane	A	cis- and trans-chlordane	cis- and trans-nonachlor, oxychlordane
3			DDT	B	p,p'-DDT, o,p'-DDT	p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDD
4			Dieldrin	A	Dieldrin	
5			Endrin	A	Endrin	Endrin ketone
6			Heptachlor	A	Heptachlor	Heptachlorepoxide
7			Hexachlorobenzene (HCB)	A + C	Hexachlorobenzene	
8			Mirex	A	Mirex	
9			Polychlorinated biphenyls (PCB)	A + C	ΣPCB_6 (6 "indicator congeners"): 28, 52, 101, 138, 153, and 180	
					PCB with TEFs[a] (12 congeners): 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189	
10			Toxaphene	A	Congeners P26, P50, P62	
11			Polychlorinated dibenzo-*p*-dioxins (PCDD)	C	2,3,7,8-substituted PCDD (7 congeners)	
12			Polychlorinated dibenzofurans (PCDF)	C	2,3,7,8-substituted PCDF (10 congeners)	
	COP-4	2009	**2. New POPs**			
13			Alpha hexachlorocyclohexane (alpha-HCH)	A	alpha-HCH	
14			Beta hexachlorocyclohexane (beta-HCH)	A	beta-HCH	

15			Gamma hexachlorocyclohexane (gamma-HCH), common name: Lindane	A	gamma-HCH	
16			Chlordecone	A	Chlordecone	
17			Pentachlorobenzene	A + C	Pentachlorobenzene	
18			Hexabromobiphenyl (HBB)	A	PBB 153	
19			Tetra- and pentabromodiphenyl ether	A	PBDE 47, 99; optional: PBDE 100	
20			Hexa- and heptabromodiphenyl ether	A	PBDE 153, 154, 175/183 (co-eluting)	
21			Perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOSF)	B	PFOS (linear and branched isomers)	
	COP-5	2011	**3. New POPs**			
22			Technical endosulfan and related isomers	A	alpha-, beta-endosulfan, endosulfan sulfate	
	COP-6	2013	**4. New POPs**			
23			Hexabromocyclododecane (HBCDD)	A	alpha-, beta-, gamma-HBCDD	
	COP-7	2015	**4. New POPs**			
24			Hexachlorobutadiene	A	HCBD	
25			Pentachlorophenol + salts	A		[Pentachloroanisole (PCA)]
26			Polychlorinated naphthalenes	A + C	[PCN *(congeners to be decided)]*	
	COP-8	2017	**5. New POPs**			
27			Decabromodiphenyl ether (DecaBDE)	A	PBDE-209	
28			Short-chain chlorinated paraffins (SCCPs)	A	[SCCP]	
(24)			Hexachlorobutadiene	C	HCBD	
	COP-9	2019	**6. New POPs**			

(continued)

Table 1 (continued)

	COP No.	Year	Parameter	Annex	Parent POPs	Transformation products
29			Dicofol		[Dicofol]	
30			Perfluorooctanoic acid (PFOA) and salts		PFOA	
			Voluntary (POPs proposed for listing)			
31			Medium-chain chlorinated paraffins (MCCP)			
32			Perfluorohexane sulfonic acid (PFHxS)			

[POP]: To be decided. Presently, the analytical methods still need further development before analytes can be recommended
[a] PCB with TEFs (toxic equivalency factors) assigned by WHO in 1998

Table 2 Time periods for the five WHO/UNEP-coordinated studies performed between 2000 and 2019 in equal 4-year intervals

	Years	Four-year intervals
3rd round	2000–2003	2000–2003
4th round	2005–2007	2004–2007
5th round	2008–2012	2008–2011
6th round	2014–2015	2012–2015
7th round	2016–2019	2016–2019

5 Assessment of Time Trends

A period of at least 10–15 years is estimated to be necessary to detect significant temporal changes of moderate size for most POPs. For example, a change of 7% per year would be necessary for a 50% decrease over 10 years. Furthermore, at least 4 to 5 years of monitoring would be necessary to give reliable estimates taking into account random within- and between-year variation and other components of variance (UNEP 2019a). In addition, the rate of decrease will vary among POPs.

This compendium with a series of publications covers five WHO/UNEP-coordinated studies of concentrations and trends of POPs in human milk, which were performed between 2000 and 2019. Furthermore, for time trends of PCB, PCDD, and PCDF, data of the first (1987–1988) and second (1992–1993) rounds are also used.

The five rounds performed between 2000 and 2019 have different lengths: The third, fourth, and seventh studies spanned periods of 4 years, the fifth of 5 years, and the sixth of 2 years. In some cases, samples were collected outside the planned time frames. However, apart from the official time frames, all samples submitted between 2000 and 2019 are included in this evaluation. Therefore, it is more appropriate to present the participation of countries and to discuss the results in equal four-year intervals as grouped in Table 2.

Assessments of temporal trends are part of the presentation of results and their discussion in Part III and specifically for countries with repeated participation in Part IV of this compendium.

6 Participating Countries and Number of Samples

6.1 Regional Distribution

Regions can be defined in various ways, among them:

- Countries and areas can be grouped geographically into six major areas designated by the United Nations as: Africa; Asia; Europe; Latin America and the Caribbean; Northern America, and Oceania (United Nations 2019a, b). This geographical approach was initially proposed by the 2007 version of the *Guidance on the Global Monitoring Plan for POPs*. For the presentation of POPs data on a regional basis, it was recommended to establish the following six regions:

Africa; the Caribbean, Central and South America; Central, Eastern, and Western Europe; Eastern, Southern, and Western Asia; North America; and the region of Australia, New Zealand, and the Pacific Islands (UNEP 2007b).
- WHO classifies its Member States into six regions (Africa, Eastern Mediterranean, Europe, Americas, South-East Asia, and Western Pacific) (WHO 2019).
- Countries can be classified according to the five United Nations geopolitical groups: the African Group, the Asia-Pacific Group, the Eastern European Group, the Group of Latin American and Caribbean Countries (GRULAC), and the Western European and Others Group (WEOG) (United Nations 2019c). Three countries that participated in the WHO/UNEP-coordinated exposure studies 2000–2019 are listed as special cases with the following attribution: Israel became a WEOG full member in 2000; Kiribati (geographically in Oceania) is not a member of any regional group despite its membership in the UN; the United States of America is not a member of any regional group, but attends meetings of the Western Europe and Other States Group (WEOG) as an observer and is considered to be a member of that group for electoral purposes.
- The Stockholm Convention's Global Monitoring Plan for POPs is implemented by the regional organization groups established in the five United Nations regions (Africa, Asia and the Pacific, Eastern Europe, Latin America and the Caribbean, Western Europe and Others). A global coordination group is in place to harmonize and coordinate implementation activities among the five UN regions.

In accordance with the implementation of the GMP, countries report flexibly through one of the five UN Regional Groups allowing more direct comparisons and conclusions regarding the regional reports for the Convention (UNEP 2007c).

6.2 Regional Participation Over Time

Figure 2 illustrates the 2020 status of global participation of countries, including their periods of participation.

Based on grouping the studies into four-year periods, a summary of the participation of countries between 2000 and 2019 and the number of pooled samples submitted are given in Table 3. Note that the Asia-Pacific Group has been subdivided to separate Asian and Pacific Islands subgroups as their exposures to POPs are quite different.

For assessments of time trends, the repeated participation in the WHO/UNEP-coordinated exposure studies is necessary. Of the 82 countries, 50 participated in two or more studies during the period 2000–2019. This includes 13 from the African Group, eight from the Asia/Pacific Group, nine from the Latin American and Caribbean Group, nine from the Eastern European Group, and 11 from the Western European and Others Group. From these 50 countries, 41 repeated twice, six three-times, and three four-times (Table 4).

Calculations of time trends are possible for those POPs, which were of interest at times of submission of the sample; for some POPs, this also depends on the sample

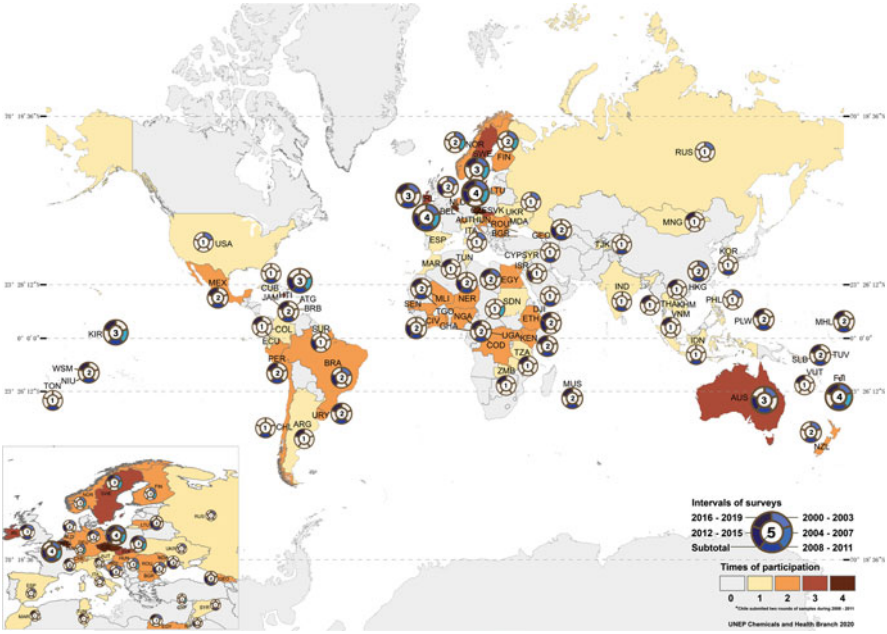

Fig. 2 Regional distribution, number of participations, and time period of participating countries 2000–2019

Table 3 Number of countries and number of pooled samples submitted for the WHO/UNEP-coordinated surveys during the five rounds performed between 2000 and 2019

Group	Countries	No. of samples	Number of countries participating in the period				
			2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
African	19	40	1	1	12	3	15
Asia-Pacific/Asia subgroup	12	29	2	1	6	0	4
Asia-Pacific/Pacific Islands subgroup	10	24	1	2	9	0	8
Latin American and Caribbean	14	36	1	1	10	3	9
Eastern European	11	43	8	3	3	7	2
Western European and Others	16	60	13	5	5	4	5
Total	82	232	26	13	45	17	43

amount submitted. As described earlier, the third round included initially only PCB, PCDD, and PCDF. Later, the scope of the exposure studies was expanded to include other POPs, as well. Therefore, all samples for the period 2000–2015 were analyzed

Table 4 Number of participations of countries during the period 2000 to 2019

Group	Once	Twice	3-times	4-times	No. of countries participating repeatedly
African	6	13	0	0	13
Asia-Pacific/Asia subgroup	11	1	0	0	1
Asia-Pacific/Pacific Islands subgroup	3	5	1	1	7
Latin American and Caribbean	5	8	1	0	9
Eastern European	2	7	1	1	9
Western European and Others	5	7	3	1	11
Total	32	41	6	3	50

for PCB, PCDD, and PCDF but a variable number for other POPs. However, the samples of the 2016–2019 round were analyzed for all 30 POPs listed as of 2019 and two POPs proposed for listing (see Sect. 4) forming a basis for evaluation of temporal trends for the recently listed POPs in future studies.

In Table 5, the 232 submitted pooled samples are differentiated with regard to the different intervals. For the third round (2000–2003), countries were encouraged to prepare two or more pooled samples. The concept was modified for the fourth round (2004–2007) and following rounds where submission of at least one representative pooled sample for countries up to a population of 50 million was required.

While 102 pooled samples were received for the third round, all but one out of 26 countries participating submitted multiple pooled samples with five countries submitting six samples or more.

6.2.1 African Group

Table 6 lists the 19 countries from the African Group combined with the indication of the periods of their participation between 2000 and 2019. A total of 13 countries participated repeatedly during this period.

All countries (except one) submitted one pooled sample and therefore, 31 of the 40 pooled samples from the African Group are considered to represent the respective country at a certain time. However, nine pooled samples from various areas of Egypt were collected between 2001 and 2002 as follow-up of findings of elevated levels of PCDD/PCDF in human milk samples from that country collected in 1997 (Malisch et al. 2000).

6.2.2 Asia-Pacific Group

Table 7 lists the 22 countries (and an area) from the Asia-Pacific Group combined with the indication of the periods of their participation between 2000 and 2019. This group is split into two subgroups, namely, 12 countries were assigned to the Asian subgroup and 10 countries to the Pacific Islands subgroup (Note that Australia and

Table 5 Number of pooled samples received from countries of the five UN regions (with split of the Asia-Pacific Group into the Asia and Pacific Islands subgroups) during the period 2000 to 2019

Group	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019	Subtotal
African	9	1	12	3	15	40
Asia-Pacific/Asia subgroup	15	1	9	0	4	29
Asia-Pacific/Pacific Islands subgroup	2	3	11	0	8	24
Latin American and Caribbean	11	1	10	5	9	36
Eastern European	28	3	3	7	2	43
Western European and Others	37	7	5	5	6	60
Total	102	16	50	20	44	232

Table 6 Countries from the African Group participating between 2000 and 2019

	Years				
	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
Congo (Dem.Rep.)			x		x
Côte d'Ivoire			x	x	
Djibouti			x		
Egypt	x				x
Ethiopia				x	x
Ghana			x		x
Kenya			x		x
Mali			x		x
Mauritius			x		x
Morocco					x
Niger			x	x	
Nigeria			x		x
Senegal			x		x
Sudan		x			
Tanzania					x
Togo			x		x
Tunisia					x
Uganda			x		x
Zambia					x
Total	1	1	12	3	15

New Zealand belong to the Western European and Others Group, see Sect. 6.2.5). One country from the Asian subgroup and seven countries from the Pacific Islands subgroup participated repeatedly during various periods.

Table 7 Countries from the Asia-Pacific Group participating between 2000 and 2019

	Years				
	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
Cambodia					x
Cyprus		x			
Hong Kong SAR, China	x		x		
India			x		
Indonesia			x		
Korea (Rep.)			x		
Mongolia					x
Philippines	x				
Syria			x		
Tajikistan			x		
Thailand					x
Vietnam					x
Number of Asia subgroup countries per round	2	1	6	0	4
Fiji	x	x	x		x
Kiribati		x	x		x
Marshall Islands			x		x
Niue			x		x
Palau			x		x
Samoa			x		x
Solomon Islands			x		x
Tonga			x		
Tuvalu			x		
Vanuatu					x
Number of Pacific Islands subgroup countries per round	1	2	9	0	8
Total number of Asia-Pacific Group countries per round	3	3	15	0	12

Most countries submitted one pooled sample for a certain round. In 2001–2002, Hong Kong SAR, China, submitted 13 samples for different risk assessment and management purposes as well as four samples in 2009. Fiji provided two samples in the years 2002, 2006, and 2011, and the Philippines in 2002. Furthermore, one sample was mixed from the small sample amounts obtained initially in 2011 from Niue, Palau, and the Solomon Islands due to a low number of donors available at that time and analyzed to get an orientation on levels. Later in 2011, sufficient sample amounts were submitted by these three countries for individual results.

6.2.3 Group of Latin American and Caribbean Countries (GRULAC)

Table 8 lists the 14 countries from the GRULAC combined with the indication of the periods of their participation between 2000 and 2019. Nine countries participated

Table 8 Countries from the Group of Latin America and the Caribbean participating between 2000 and 2019

	Years				
	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
Antigua-Barbuda			x		x
Argentina					x
Barbados			x		x
Brazil	x			x	
Chile			2 x		
Colombia					x
Cuba			x		
Ecuador					x
Haiti		x	x	x	
Jamaica			x		x
Mexico			x		x
Peru			x		x
Suriname				x	
Uruguay			x		x
Total	1	1	10	3	9

repeatedly during this period. All countries except Brazil submitted one sample in a particular period. Because of its large size and high population (over 200 million), Brazil provided 10 pooled samples from various regions that were prepared in 2001–2002 and three pooled national samples that were prepared in 2012. Samples from Chile were received in 2008 and 2011.

6.2.4 Eastern European Group

Table 9 lists the 11 countries from the Eastern European Group combined with the indication of the periods of their participation between 2000 and 2019. Nine countries participated repeatedly during this period.

Multiple samples were submitted only in the period 2000–2003 by eight countries. With regard to its large size and high population (more than 140 million), seven pooled samples from various regions of Russia were collected during 2001–2002. The other seven countries submitted between two and four samples each.

6.2.5 Western European and Others Group (WEOG)

Table 10 lists the 16 countries from the WEOG combined with the indication of the periods of their participation between 2000 and 2019. Eleven countries participated repeatedly in this period.

Twelve countries provided more than one sample in certain sampling periods for a total of 60 pooled samples. Two countries indicated that certain samples were not representative for the whole country, namely, Belgium in 2010 providing a sample from Flanders, and Sweden in 2001 providing a sample from Uppsala County.

Table 9 Countries from the Eastern European Group participating between 2000 and 2019

	Years				
	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
Bulgaria	x			x	
Croatia	x			x	
Czech Republic	x	x		x	x
Georgia			x	x	
Hungary	x	x			
Lithuania			x	x	
Moldova			x	x	
Romania	x			x	
Russia	x				
Slovak Republic	x	x			x
Ukraine	x				
Total	8	3	3	7	2

Table 10 Countries from WEOG participating between 2000 and 2019

	Years				
	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
Australia	x		x	x	
Austria					x
Belgium	x	x	x	x	
Finland	x	x			
Germany	x				x
Ireland	x		x		x
Israel				x	
Italy	x				
Luxembourg	x	x			
Netherlands	x			x	
New Zealand	x		x		
Norway	x	x			
Spain	x				
Sweden	x	x			x
Switzerland			x		x
USA	x				
Total	13	5	5	4	5

7 Summary and Conclusions

Biomonitoring of POPs can assess integrated human exposure, occurring mainly through foods, and reflects body burdens of POPs, which is due to their long half-lives. The collection of human milk is a non-invasive sampling method and thus, has many practical and procedural advantages over the collection of other biological

samples, such as blood or adipose tissue. Furthermore, from an analytical point of view, the relatively high fat content of human milk (about 4%) makes the extraction of sufficient amounts of lipids easier. These aspects in combination with the use of large volume pooled (composite) samples (often over 1 l), considered to represent a country or a subgroup at the time of sampling, have significant advantages: (1) considerably reduced costs, (2) simplified logistics, (3) lowered limits of quantification, (4) improved precision of measurements, and (5) possibility to apply various determination methods for the total of 30 chemicals in the 2019 Stockholm Convention list. In regard to the last point, for the application of various analytical methods for determination of this high number of different analytes, large sample volumes are necessary as no multi-residue method exists that would allow the simultaneous determination of all analytes of interest.

The development of WHO/UNEP-coordinated exposure studies between 1987 and 2019 is illustrated in Fig. 3. The first two rounds were performed at the end of the 1980s and the beginning of the 1990s with the determination of PCB, PCDD, and PCDF. With the same analytes, the third round started in 2000. After the adoption of the Stockholm Convention on Persistent Organic Pollutants in 2001 and its entering into force in 2004, the number of analytes of interest was expanded to cover the initial 12 POPs.

In 2007, the Conference of the Parties to the Stockholm Convention adopted the Global Monitoring Plan for POPs. Monitoring of human milk was identified as a key element for the effectiveness evaluation of the Convention. Repeated participation of countries in exposure studies allows the assessment of time trends.

2001: Stockholm Convention adopted

2004: Stockholm Convention entry into force

From 2007: Stockholm Convention Global Monitoring Plan implementation

Conference of the Parties			2001		2009	2011	2013	2015	2017	2019
No of POPs			12		21	22	23	26	28	30

Round	1	2	3	4	5	6	7
Years	1987-88	1992-93	2000-03	2005-07	2008-12	2014-15	2016-19
Coverage of POPs							
Initial focus	PCB, PCDD/PCDF	PCB, PCDD/PCDF	PCB, PCDD/PCDF	12 POPs	12 POPs	23 POPs	23 POPs
Later extended to			21 POPs	21 POPs	22 POPs		32 POPs (30 listed, 2 proposed for listing)
Submission of samples in period *)	1987-88	1992-93	2000-03	2004-07	2008-11	2012-15	2016-19
N (countries)	19	19	26	13	45	17	43
Region	Asia: 4	Asia: 1	Africa: 1	Africa: 1	Africa: 12	Africa: 3	Africa: 15
	EEG: 3	EEG: 8	Asia: 2	Asia: 1	Asia: 6	Asia: 0	Asia: 4
	WEOG: 12	WEOG: 10	Pacific: 1	Pacific: 2	Pacific: 9	Pacific: 0	Pacific: 8
			GRULAC: 1	GRULAC: 1	GRULAC: 10	GRULAC: 3	GRULAC: 9
			EEG: 8	EEG: 3	EEG: 3	EEG: 7	EEG: 2
			WEOG: 13	WEOG: 5	WEOG: 5	WEOG: 4	WEOG: 5

*) equal four-years-intervals in period 2000 - 2019

Fig. 3 Overview of WHO- and UNEP-coordinated exposure surveys between 1987 and 2019 with expansion of number of POPs and number of participating countries in the five UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; GRULAC = Group of Latin American and Caribbean Countries; EEG = Eastern European Group; WEOG = Western European and Others Group)

In addition to the number of POPs listed in the Convention at the time of the study, proposed new anticipated POPs were also included. Therefore, the third round was started in 2000 with focus on PCB, PCDD, and PCDF but was expanded after the adoption of the Convention in 2001 to cover all initial 12 POPs. In addition, 9 more POPs were included, which were later listed by the Convention. Therefore, data for 21 Convention POPs were already available for the samples of the third round (2000–2003) analyzed as a pilot study. Similarly, when the seventh round started in 2016, 23 chemicals were required to be analyzed. In order to have data on the complete picture of the 30 chemicals listed until 2019 (28 chlorinated or brominated, 2 perfluorinated), it was decided to cover the additional new POPs, furthermore two chemicals proposed for listing under the Convention. By this expansion, the first data for new POPs are provided as starting point for future effectiveness evaluations.

Whereas in the beginning more countries from the Eastern European Group and the Western European and Others Group participated in these exposure surveys, since 2008 more countries from Africa, Asia and the Pacific, and the Group of Latin American and Caribbean Countries participated. These countries were often supported by GEF-financed projects. Altogether 82 countries participated and 50 of these participated repeatedly, which resulted in 144 participations among the five studies performed between 2000 and 2019. This is broken down as follows: 32 from the African Group, 33 from the Asia-Pacific Group, 24 from the Group of Latin American and Caribbean Countries, 23 from the Eastern European Group, and 32 from the Western European and Other States Group.

Acknowledgments This publication was developed by CVUA Freiburg, Germany, in the framework of the UNEP/GEF projects titled “Implementation of the POPs Monitoring Plan in the Asian Region” and “Continuing Regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region.”

The substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk studies, is greatly appreciated. Further, the worldwide implementation of the Global Monitoring Plan for POPs, including that of the WHO/UNEP global human milk studies, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden and through the European Union’s Global Public Goods and Challenges Programme (GPGC).

The authors express their gratitude to the National Coordinators of the WHO/UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the WHO/UNEP Reference Laboratories for the human milk survey, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the reference laboratories was important for the successful organization of these studies on a global level.

Hae Jung Yoon, Seongsoo Park, and Philippe Verger (Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

We thank Katarina Magulova (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Haosong Jiao and Gamini Manuweera (UNEP Economy Division, Chemicals and Health Branch) for their support and contributions to these studies, and specifically to Heidelore Fiedler

for the conception and implementation of the Global Monitoring Plan projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

The data belong to WHO and/or UNEP and countries and are publicly available in the Data Warehouse of the Stockholm Convention Global Monitoring Plan (GMP DWH).

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The Stockholm Convention, Global Monitoring Plan and its Implementation in Regional and Global Monitoring Reports

Kateřina Šebková

Abstract

The Stockholm Convention on Persistent Organic Pollutants (POPs) is a global legally binding agreement focusing on the protection of health and the environment from negative impact of listed manmade chemicals, persistent organic pollutants. The Convention required the development and regular update of National Implementation Plans, periodic national reporting (Art. 15), and the establishment of other expert frameworks for the assessment of candidate chemicals (Persistent Organic Pollutants Review Committee [POPRC]). For the evaluation of the effectiveness (Art. 16) it was required to develop a mechanism to identify and compile and/or a mechanism capable of generating coordinated, harmonized, and validated information on changes in levels of its target chemicals over time. The Global Monitoring Plan (GMP) is the mechanism put in place in 2007 that so far produced three sets of regional monitoring reports containing current findings on POPs concentrations in individual UN regions and subsequently, three global reports synthesizing the available information on the global scale. POPs data reported by the Regional Organization Groups (ROGs) in these reports are key pillars and inputs into the effectiveness evaluation that periodically assesses outputs of measures adopted in the Stockholm Convention.

Activities under the GMP are governed by its implementation plan, supported by the technical Guidance on the Global Monitoring Plan and overseen by experts organized in the Regional Organization Groups and in the Global Coordination Group. As the Stockholm Convention expands its scope over time, it is necessary to continuously update the technical knowledge and guidance but also to ensure sustainability of POPs monitoring activities. Availability and continuity of

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_3

long-term POPs monitoring programmes and their data is decisive for global decision-making.

Keywords

Stockholm Convention on Persistent Organic Pollutants · Global Monitoring Plan · Effectiveness evaluation · Core media · Regional Organization Group · Guidance on the Global Monitoring Plan for POPs · Conference of the Parties

1 Background Information on the Stockholm Convention

The Stockholm Convention on Persistent Organic Pollutants (POPs) is a global legally binding agreement focusing on the protection of health and the environment from negative impact of listed manmade chemicals, persistent organic pollutants. The Convention was adopted in 2001 (UNEP 2001), entered into force in 2004 and during its nine decision-making meetings of the Conference of the Parties (COP) to the Stockholm Convention in 2005, 2006, 2007, 2009, 2011, 2013, 2015, 2018, and 2019 (UNEP 2001–2021) expanded the number of chemicals covered by the Convention from twelve chemicals listed initially to thirty as of early 2020 (see footnote 1–3 below).

These target POPs are classified into three groups defined by their use, however some of the chemicals fit into more than one of these three general categories:

- pesticides used in agricultural applications [1]
- industrial chemicals used in various applications [2]
- chemicals generated unintentionally as a result of incomplete combustion and/or chemical reactions [3]

The footnotes below show for each POP the name, abbreviation and indicate meetings of the Conference of the Parties at which the listing of the chemicals took place. Those shown in bold font are originally listed POPs at the adoption of the convention.

[1] **aldrin**, **chlordane**, chlordecone (COP-4, 2009), **dichlorodiphenyltrichloroethane (DDT)**, dicofol (COP-9, 2019), **dieldrin**, endosulfan (COP-5, 2011), **endrin**, **heptachlor**, **hexachlorobenzene (HCB)**, gamma-hexachlorocyclohexane (γ-HCH, lindane) and by-products of lindane [alpha-hexachlorocyclohexane (α-HCH) and beta-hexachlorocyclohexane (β-HCH)] (COP-4, 2009), pentachlorophenol, its salts and esters (COP-7, 2015), **mirex, toxaphene**.

[2] tetra- and pentabromodiphenyl ethers (PBDE) (COP-4, 2009), hexa- and heptabromodiphenyl ethers (PBDE) (COP-4, 2009), decabromodiphenyl ether (COP-8, 2017), hexabromocyclododecane (HBCD) (COP-6, 2013), hexabromobiphenyl (COP-4, 2009), hexachlorobutadiene (COP-7, 2015), perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOS-F) (COP-4, 2009), perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds (COP-9, 2019), pentachlorobenzene (PeCB) (COP-4, 2009), **polychlorinated biphenyls (PCB)**, polychlorinated naphthalenes (PCN) (COP-7, 2015), short-chain chlorinated paraffins (SCCPs) (COP-8, 2017).

[3] **hexachlorobenzene (HCB)**, hexachlorobutadiene (COP-8, 2017), pentachlorobenzene (PeCB) (COP-4, 2009), polychlorinated naphthalenes (PCN) (COP-7, 2015), **polychlorinated biphenyls (PCB)** and **polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF)**.

The objective of the Stockholm Convention on POPs is to protect human health and the environment from POPs. Therefore, the Convention required the development and regular update of National Implementation Plans, periodic national reporting (Art. 15) and the establishment of other expert frameworks for assessment and listing of new chemicals, exemptions and Best Available Techniques/Best Environmental Practices (BAT/BEP). Furthermore, reviews on the progress in eliminating PCB, DDT, and other chemicals from production and use are required.

Moreover, to assess the outcomes of measures implemented, the Convention also contains requirements related to the collection of information on the presence and movement of target chemicals in the environment as well as harmonized and validated information on changes in their levels over time. Indeed, the Article 16 of the Stockholm Convention on effectiveness evaluation requires the Conference of the Parties to evaluate periodically whether the Convention is an effective tool in achieving the objective of protecting human health and the environment from persistent organic pollutants. Such evaluation is based a) on reports and other monitoring information that is comparable and covers the presence of listed POPs as well as their regional and global environmental transport, b) on information from the national reports under Article 15, and c) non-compliance information under Article 17, as stated in the paragraph 3 of the Article 16.

For the purposes of this compendium, we will focus only on the matters relevant to the POPs monitoring.

2 Development of Arrangements for POPs Monitoring

This section contains the steps and arrangements that lead to generation of the POPs monitoring reports under the Stockholm Convention describing data on POPs identified by the ROGs.

The Stockholm Convention text requires that at its first meeting the Conference of the Parties (COP) initiates the establishment of arrangements to provide itself with comparable monitoring data on the presence of listed POPs and further specifies criteria for the arrangements: to draw on existing knowledge and activities, to be organized regionally, and to regularly produce reports for consideration by the COP (UNEP 2001, paragraph 2 of Article 16).

Thus, at the first meeting in 2005, the COP adopted the "Proposal for arrangements to provide the Conference of the Parties of the Stockholm Convention with comparable monitoring data on the presence of the chemicals listed in Annexes A, B and C of the Convention" (UNEP 2005a, UNEP/POPS/COP.1/21, Annex II), decided to field test/pilot the proposed arrangements, and prepare a proposal for global monitoring plan (UNEP 2005b, decision SC-1/13) by drawing on additional materials (UNEP 2003).

At the next meeting in 2006, the COP endorsed that the first POPs monitoring report will provide baselines for further evaluations, air monitoring and human exposure through breast milk or blood serum used as core data, that POPs monitoring data are needed to cover all five UN regions, and that an establishment of strategic arrangements and partnership including with the health sector was required. Furthermore, that decision stipulated that the global monitoring plan under preparation should be practical, feasible, and sustainable, achieve global coverage, contain at least core representative data from all regions, be designed to go beyond the first monitoring report, and address long-term needs for receiving appropriate representative data in all regions (UNEP 2006, decision SC 2/13). In addition, the elaboration of a guidance document on standardization of monitoring approaches was also requested in UNEP 2006. A provisional ad hoc technical working group was given the task to prepare these documents and to coordinate the implementation of the preliminary global monitoring plan.

At its meeting in 2006, the COP agreed to complete the first effectiveness evaluation in 2009. This timeline meant that the first POPs monitoring reports needed to be available as well by 2009 at the latest.

Thus, the third meeting of the Conference of the Parties held in Dakar, Senegal in May 2007, considered the first draft of the Guidance on the Global Monitoring Plan for Persistent Organic Pollutants (UNEP 2007b) and adopted the amended Global Monitoring Plan for Persistent Organic Pollutants for Effectiveness Evaluation on a provisional basis (UNEP 2007a), and established regional organization groups and a global coordination group (UNEP 2007c, decision SC 3/19) to prepare the regional and global reports for consideration at the COP meeting in 2009. The work was driven by and based on the contributions of the Regional Organization Groups (ROGs) and their work identifying and compiling the best available information on levels of listed POPs in core media (ambient air and human milk and blood samples). The POPs data compiled by the ROGs are the result of the work of a number of strategic partners and monitoring programmes performing POPs monitoring at global, regional, and national scale including the World Health Organization (WHO), UN Environmental Programme-Global Environment Facility monitoring projects (UNEP-GEF), Arctic Monitoring and Assessment Programme (AMAP), Great Lakes of NA (*North America*), Northern Contaminants Programme (NCP), European Monitoring and Evaluation Programme (EMEP), Toxic Organic Micro-Pollutants network (TOMPs), Ospar, Helcom, Global Atmospheric Passive Sampling network (GAPS), Monitoring Network of ambient air by passive sampling (MONET), Latin American Passive Atmospheric Sampling Network (LAPAN), East Asia Network and others that provided the information to the ROGs to develop the first set of GMP regional reports. On the basis of the first set of regional POPs monitoring reports adjustments of the Global Monitoring Plan were adopted in 2009 as described in the next section.

3 Organizational Framework for POPs Monitoring and its Further Development

The fourth meeting of the COP to the Stockholm Convention confirmed the following three components as the pillars of the Global Monitoring Plan framework (UNEP 2009a): expert groups, updated technical guidance document on the Global Monitoring Plan and GMP Implementation Plan. All three components are described in more detail below:

3.1 Expert Groups

A global group is responsible for coordinating the activities at global and regional levels—*Coordination Group* (15 members, 3 per UN region). This group oversees global monitoring activities and is responsible for development of the global POPs monitoring report submitted to the effectiveness evaluation process in each phase. The mandate of the group has been updated after completion of the effectiveness evaluation (UNEP 2017a).

At regional level experts are organized in five *Regional Organization Groups* (ROG). Each UN region has one ROG consisting of six members elected by governments from the region. ROG members oversee and coordinate POPs monitoring activities in their region, are responsible for the preparation of the regional monitoring reports, and communicate with all regional stakeholders from governments, monitoring programmes, data providers, researchers, regional centres as well as with the Secretariats of the Basel, Rotterdam and Stockholm conventions (BRS Secretariat). The mandate of the ROG has been updated after completion of the effectiveness evaluation (UNEP 2017a).

3.2 Guidance Document

The second pillar is the *technical guidance document on the Global Monitoring Plan* that is continuously updated to gather current expert knowledge on POPs data generation and collection including selection of core matrices, sampling, sample processing, chemical analyses, statistical considerations as well as storage of data on POPs and their reporting (UNEP 2007b, 2009a and 2021b).

3.3 GMP Implementation Plan

The third pillar is the frequency and scope of preparing the regional reports that has been set to six-year intervals by the *GMP Implementation Plan* (UNEP 2009a, 2009b).

Over time, updates of the framework have taken place.

Firstly, with listing of the new industrial chemicals in 2009, *surface water* was added as additional core medium for hydrophilic POPs (perfluoroctanesulphonate—PFOS), as explained in the Chaps. 2 and 4 of the updated guidance document in 2013 (UNEP 2013c).

Secondly, at its sixth meeting in May 2013, the Conference of the Parties, by decision SC-6/23 on the GMP for the effectiveness evaluation, adopted the amended *GMP for POPs* (UNEP/POPS/COP.6/INF/31/Add.1) and the amended *implementation plan for the GMP* (UNEP/POPS/COP.6/INF/31/Add.2) (UNEP 2013a, 2013b). The implementation plan was further amended in 2017 (UNEP 2017d).

Thirdly, the Guidance document for POPs monitoring is also continuously updated due to the expansion of the scope of the Convention as well as by gathering more relevant experience and knowledge on scientific advances in sampling, analyses, interpretation, and storage of samples and data. At COP-10, the latest version of the updated Guidance (UNEP/POPS/COP.10/INF/42), which addresses the sampling and analysis of POPs added to the Convention in 2017 and 2019, was made available. The guidance also comprises a detailed chapter on POPs monitoring in human milk including the online version of the WHO sampling protocol (Annex 3) pertaining to the milk survey (UNEP/WHO 2009) described in detail in Chap. 1 (Fürst, 2023) and Chap. 2 (Malisch et al. 2023) of this part of the compendium.

4 Outputs of the Global Monitoring Plan

4.1 Regional and Global POPs Monitoring Reports

The GMP produces reports at every six years cycle (UNEP 2009a, 2009b). The interval length and timing has been further aligned with the effectiveness evaluation cycle by amendment to the implementation plan for the GMP (UNEP/POPS/COP.6/INF/31/Add.2) endorsed by the decision SC-6/23 at the sixth meeting of the COP in 2013 (UNEP 2013a).

The first set of regional POPs monitoring reports produced for COP4 in 2009 provided information on changes in concentrations of the 12 POPs initially listed in the Stockholm Convention (UNEP 2011a). The second set of regional POPs monitoring report endorsed in 2015 contained information on changes in concentrations and trends of the 12 POPs initially listed in the Stockholm Convention and information on baseline concentrations of the 11 substances newly listed in the annexes to the Convention in 2009, 2011, and 2013 (UNEP 2015). The third set of regional reports synthesize information from previous regional reports and covers both the full scope of the chemicals listed in the Convention as of 2019 and some candidate POPs. Due to longer time series, the ability to derive time trends for different listed POPs increases. These reports were published in spring 2021 (UNEP 2021a).

As demonstrated above, the scope of the regional report is gradually expanding and covers all POPs listed in the Stockholm Convention Annexes. The main focus of the report is the assessment of datasets in the core media—ambient air, human tissues (human breast milk or blood), and water for hydrophilic POPs, but other

media such as soil, biota, plants are also used to support interpretation of observed levels and their trends.

The reports have a uniform structure and contain a number of visual outputs, usually generated from the electronic data warehouse of the Global Monitoring Plan (GMP DWH). These maps, charts, and tables illustrated POPs data availability, time trends, or summary information at regional or global scale. See, for example, the report of Central and Eastern European Region (CEE report) in 2015 (UNEP 2015) or the second global report 2017 (UNEP 2017c).

Once regional reports are released, the Coordination Group prepares a global report synthesizing POPs monitoring information on levels, trends, and long-range transport at the global level on the basis of individual regional reports. The resulting global GMP report serves as key input into the effectiveness evaluation process. There were so far three sets of global reports produced—a pilot report in 2009, the second report in 2017 (UNEP 2017c) and the third report was produced for COP11 held in 2023 (UNEP 2023).

The regional and global monitoring reports are available at the homepage of the Stockholm Convention as shown in UNEP (2021a).

4.2 Improved Interoperability and Access to Global POPs Data in Electronic Format

After the first set of the regional reports was released in pdf format and experiencing the challenges in producing the first global report for the pilot effectiveness evaluation by compiling information contained in five separate regional reports produced in a different manner, a new user-friendly solution for POPs data management and visualization was sought/needed (UNEP 2011b). In preparation for the second effectiveness evaluation phase, the Stockholm Convention Secretariat has requested a content analysis of information available in the first set of regional POPs monitoring reports focusing on initial 12 POPs (GMP et al. 2014) and their conversion into a pilot online platform able to compiling and archiving GMP POPs data in regional data repository to support work of the regional organization groups and facilitate production of regional and global reports. Under the supervision of the GMP Coordination Group an online tool to compile, harmonize, archive, and visualize available POPs data was established at www.pops-gmp.org to help and improve the POPs data flow between and within the ROGs and facilitate the production of consistent reports from the data the ROGs have compiled.

The platform was developed in a strict conformity with Chap. 6 of the Guidance on the Global Monitoring Plan for Persistent Organic Pollutants relevant to data handling and respecting the scope and parameters and supplementary information for target chemicals in core matrices. Further improvements were made after the pilot and the electronic database and visualization platform, GMP Data Warehouse has been made operational during the second GMP phase, supporting the regional organization groups in the work for the assembling, processing, storing, and presentation of monitoring data for the second set of regional reports and the global report

in 2015 and 2017, respectively. Special focus was on interoperability, access, transparency, and QA/QC in stable, curated data repositories trusted by data providers.

Prior 2019, the GMP Data Warehouse was further developed, with new data visualization tools added to continue to assist the ROGs and the Coordination Group in producing the consistent regional and global monitoring reports in 2021 and beyond (GMP et al. 2020).

The global monitoring plan and the ROGs within their mandate produced significant POPs data compilations in the three phases to date. Experience shows that it is crucial to continue to network effectively with existing data repositories, avoid duplication, and adapt to changes in analytical and computing tools.

Currently, the GMP data warehouse ensures interoperability and cooperation between various data repositories and contains modules that avoid duplication of datasets and enhance transparency, access, and QA/QC standards. The datasets on the portal get updated every six years, in line with the timeline of preparation of regional reports.

Last but not least, the online visualization of the GMP data warehouse also constitutes a publicly available portal once the regional reports are endorsed and shows the largest global POPs datasets in core matrices including the human milk POPs data. POPs data reported by the ROGs are key pillars and inputs into the effectiveness evaluation that periodically assesses outputs of measures adopted in the Stockholm Convention.

5 Effectiveness Evaluation

As discussed above in the background section of this chapter, Article 16 uses the outputs of the Global Monitoring Plan, namely the global monitoring report as one key input into the review of information on whether the Stockholm Convention is effective in achieving its objective.

The first pilot effectiveness evaluation of the measures set by the Stockholm Convention was undertaken at COP4 in 2009, and the first full effectiveness evaluation cycle was completed at COP8 in 2017 (UNEP 2017b).

That effectiveness evaluation report in evaluating the Global Monitoring Plan provided the following conclusions and recommendations to the Stockholm Convention stakeholders in relation to POPs monitoring activities continuity, sustainability, and usefulness (UNEP 2017b):

> It is of great importance that the global, regional and national programmes evaluating time trends of both POPs and other environmental pollutants in blood and/or milk continues. This data is needed to be able to follow the effectiveness of the Stockholm Convention and to see that regulations and other actions taken in order to reduce the exposure to POPs are purposive and efficient. The UNEP/WHO Human Milk Survey

(continued)

could be continued with timing better synchronized with the cycle of the effectiveness evaluation of the Stockholm Convention to enable the GMP to use the latest available data. Participation from more countries in this survey would increase its representativeness;
Monitoring should be continued and expanded for newly listed POPs to provide the information needed to assess changes over time. In order to be able to follow up on these substances over time, it is important to start monitoring these substances now. And, in addition to measuring the classic POPs, that are already regulated, it is important to also monitor possible substitution substances;
Archiving of human samples should be encouraged as a cost-effective means for conducting retrospective analysis for newly listed POPs, to generate baseline information and time trends for new pollutants as they are added to the Convention.

As quoted above, the effectiveness evaluation results also influence the direction and content/scope of activities under the Global Monitoring Plan. In the effectiveness evaluation the implementation of the GMP is weighted from the holistic perspective and on the capacity to fulfil the needs of the Stockholm Convention. As demonstrated above, the Global Monitoring Plan's continuous and sustainable implementation is a prerequisite for the effectiveness evaluation of the Convention.

Currently, a third effectiveness evaluation cycle started by establishing the Effectiveness Evaluation Committee to produce the documents for COP 11 in 2023 (UNEP 2019 (SC-9/17) and UNEP 2021a).

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Part II

Analytical Methods and Quality Control

Analysis and Quality Control of WHO- and UNEP-Coordinated Human Milk Studies 2000–2019: Polychlorinated Biphenyls, Polychlorinated Dibenzo-*p*-dioxins, and Polychlorinated Dibenzofurans

Rainer Malisch and Alexander Schächtele

Abstract

The analytical method used for the determination of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) in human milk comprised extraction of lipids, the use of 51 $^{13}C_{12}$-labelled PCB and PCDD/PCDF as internal standards, several chromatographic purification steps, and high resolution gas chromatographic/high resolution mass spectrometric measurement. As an accredited laboratory since 1998, a comprehensive quality control programme has been applied to assure the long-time reliability of results of human milk samples received for WHO/UNEP-coordinated exposure studies between 2000 and 2019. This included procedural blanks, the use of fortified vegetable oil and numerous quality control samples as an in-house reference material, duplicate analyses, and successful participation in 32 proficiency tests (PTs) covering 81 samples of food of animal origin or human milk. Trueness was estimated from the PT samples in the relevant range for human milk above 1 pg WHO-TEQ/g lipid: The deviation was less than 10% from the assigned values for WHO-PCDD/PCDF-PCB-TEQ and WHO-PCDD/PCDF-TEQ and less than about 15% for WHO-PCB-TEQ for about 90% of the results. For the sum of six non-dioxin-like PCB (relevant occurrence range, 1–1000 ng/g lipid), approximately 90% of the results differed by less than 15% from the assigned values. A long-term precision of $<15\%$ (coefficient of variation of within-laboratory reproducibility) was achieved, based on quality control samples analysed between 2000 and 2019.

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_4

The analytical methodology used fulfilled the requirements of the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025, the analytical criteria for PCDD/PCDF and PCB in feed and food specified in EU legislation, and the criterion for monitoring information for Parties to the Stockholm Convention.

Keywords

Analysis · Polychlorinated biphenyls (PCB) · Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDF) · Quality control · Trueness · Long-term precision · Human milk · Global WHO/UNEP studies

1 Introduction

This compendium comprises a series of articles, among them the overview of the World Health Organization (WHO) and the United Nations Environment Programme (UNEP)-coordinated exposure studies on persistent organic pollutants (POPs) in human milk and their link to the Stockholm Convention (Malisch et al. 2023a); the findings and discussion of results of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) (Malisch et al. 2023b); and the assessment of time trends derived from countries with repeated participation for PCB and PCDD/PCDF (Malisch et al. 2023c).

The references used for the review on findings of POPs in human milk (Fürst 2023) show a wide range of analytical methods for determination of PCB and PCDD/PCDF. This chapter describes the analytical methods and quality control used for the determination of PCDD/PCDF, dioxin-like polychlorinated biphenyls (DL-PCB), and non-dioxin-like polychlorinated biphenyls (NDL-PCB) in human milk samples obtained from the WHO/UNEP-coordinated exposure studies performed between 2000 and 2019.

2 Materials and Methods

The protocols for the collection of samples, preparation of pooled samples considered to be representative for a country or a subgroup and their submission to dedicated Reference Laboratories (for chlorinated and brominated POPs between 2000 and 2019: CVUA Freiburg, Germany) and the overview on the participating countries with respect to regional distribution and the temporal differentiation of the collected samples are given in Part I (Malisch et al. 2023a).

2.1 Analytical Procedure and Analytes

After freeze-drying 100 ml human milk sample, the lipid portion containing the contaminants of interest was extracted for eight hours with ethanol/toluene (70/30, v/v), using a hot extraction device (Twisselmann extractor). After evaporation of the solvent, polar co-extractives in the crude extract were removed by dissolving the residue in tert-butyl methyl ether and re-evaporating to provide a purified fat extract.

2.5 g aliquot of this extract was spiked with $^{13}C_{12}$-labelled standards. Tables 12–16 (in the appendix) list the 55 determined native congeners and the 51 $^{13}C_{12}$-labelled standards that were used, as follows:

- Table 12: All 17 native and $^{13}C_{12}$-labelled PCDD/PCDF with 2,3,7,8-chlorine substitution (therefore with attribution of Toxic Equivalency Factors [TEF], see Sect. 2.2 "Toxic Equivalents [TEQ]")
- Table 13: 5 native and $^{13}C_{12}$-labelled non-ortho substituted PCB, which includes the 4 dioxin-like congeners with attribution of TEFs
- Table 14: 17 native and 10 $^{13}C_{12}$-labelled mono-ortho substituted PCB (including the 8 dioxin-like mono-ortho PCB with attributed TEFs)
- Table 15: 16 native and 11 $^{13}C_{12}$-labelled di/tri/tetra-ortho substituted PCB (including the 6 non-dioxin-like PCB), see Sect. 2.3 "Sum parameter for non-dioxin-like PCB ("indicator PCB")"
- Table 16: 8 $^{13}C_{12}$-labelled PCDD/PCDF without 2,3,7,8-substitution, used as recovery standards

To the lipid aliquot, the $^{13}C_{12}$-labelled standards listed in Tables 12–15 were added as **internal standards** for calculation of the recoveries for these congeners. The $^{13}C_{12}$-labelled PCDD/PCDF listed in Table 16 (without 2,3,7,8-substitution) were used as **recovery standards** (also called "injection standards"; for extract reconstitution before GC/HRMS analysis) and added after clean-up before the final determination step.

Gel permeation chromatography on Bio Beads S-X3 was used to remove fat (in four runs with a maximum of 0.75 g fat each; 50 g Bio Beads S-X3; eluent ethyl acetate/cyclohexane [1/1, v/v]). Small amounts of remaining lipid and oxidizable substances were removed using a mixed column loaded with layers of 1 g sulfuric acid (96%) impregnated silica gel and 1 g NaOH-impregnated silica gel (eluent: 20 ml heptane). A Florisil™ column (deactivated with 3% water) was used to separate the PCB (eluted with heptane containing 0.2% of toluene; as the first fraction) from the PCDD/PCDF (eluted with toluene; as the second fraction). The PCDD/PCDF-fraction was purified on a Carbopack B-column (automated version; Carbopack B/Celite mixture; the first fraction eluted with hexane contained potentially interfering substances, the second fraction was a reverse elution of the PCDD/PCDF with toluene) or on a Carbopack C-column (manual version; Carbopack C/Celite mixture; washed with heptane; then eluted with toluene).

The second fraction was evaporated to a final volume of 20 µl after the addition of the recovery standards. Initially only 1,2,3,4-$^{13}C_{12}$-TCDD was used, but since 2018,

other $^{13}C_{12}$-labelled PCDD/PCDF without 2,3,7,8-substitution have been used as additional recovery standards (see Table 16) and were the most recent improvement to the study during 2016–2019.

The PCB eluted in the first fraction were further separated into three fractions by elution through a Carbopack B-column, firstly with hexane to yield di/tri/tetra-ortho PCB, then with hexane/toluene (92.5/7.5, v/v) for the mono-ortho PCB and finally reverse eluted with toluene to yield the non-ortho PCB. Since 2016, the separation of the PCB was improved to yield just two fractions, the first of which contained all the ortho-substituted PCB, with non-ortho-substituted PCB in the second fraction. After addition of $^{13}C_{12}$-PCB 80 as recovery standard, the fractions were evaporated to a final volume of 60 µl (non-ortho PCB) or 500 µl (ortho PCB).

The measurements for these three groups of analytes (PCDD/PCDF, non-ortho PCB, and ortho PCB) were carried out using HRGC/HRMS (initially on a Fisons Autospec, later on a Thermo Fisher MAT95XP and Thermo Scientific DFS) at a resolution 10,000, and quantified against a 5-point calibration curve. PCDD/PCDF (PTV; 5 µl injection volume) were separated on a DB5-MS GC column, whereas the PCB (1 µl splitless injection) were separated using either one of STX-500 (Crossbond® carborane/dimethyl polysiloxane; Restek), HT-8 PCB (8% Phenyl-Polycarboran-Siloxan; SGE/Trajan ™), or MXT-500 (Crossbond® carborane/dimethyl polysiloxane; Restek) columns.

2.2 Toxic Equivalents (TEQ)

Toxic Equivalents (TEQ) were calculated as the sum of the products of the concentration of each compound (17 PCDD/PCDF congeners with 2,3,7,8-substitution and 12 dioxin-like PCB congeners) multiplied by the corresponding toxic equivalency factors (TEF), and provided an estimate of the summed 2,3,7,8-TCDD-like activity for both analyte groups.

The TEF values for PCDD, PCDF, and DL-PCB that were initially proposed by the 1997 WHO expert group for calculation of WHO-TEQs (Van den Berg et al. 1998) were used for the results obtained during the first two sampling periods (2000–2003; 2004–2007). These TEFs were re-evaluated at a further WHO expert meeting held in 2005 (Van den Berg et al. 2006) with changes to some values, and these revised values were used for WHO-TEQ calculations for the later sampling periods. In order to facilitate comparison with other human milk surveys (in particular with previous studies, thus allowing conclusions on time trends), the results have been calculated using both sets of TEF values and these are compared in Part III (Malisch et al. 2023b).

Three summarizing parameters can be calculated: “WHO-PCDD/PCDF-TEQ” comprising PCDD/PCDF, “WHO-PCB-TEQ” for dioxin-like PCB, and “Total TEQ” or “WHO_{2005}-TEQ” comprising PCDD/PCDF and dioxin-like PCB.

2.3 Sum Parameter for Non-dioxin-like PCB ("Indicator PCB")

Concentrations of NDL-PCB are expressed as the sum of six NDL-PCB ("indicator PCB"; ΣPCB_6) including the congeners PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180 (UNEP 2019).

3 Development of Regulations and Standards for Analytical Criteria over Time

The determination of PCDD/PCDF and PCB in the human milk samples has followed the analytical criteria for these analytes in food and feed as specified in EU legislation since 2002 (European Commission 2002a), and for the target error as required by the Global Monitoring Plan (GMP) (UNEP 2013; UNEP 2019).

3.1 Historical Background (2000–2001)

In the absence of internationally harmonized analytical criteria for the determination of PCDD/PCDF and PCB, a comprehensive quality control programme was initiated at the start of the third round of WHO/UNEP-coordinated exposure studies in 2000 (Malisch and van Leeuwen 2002). In 2001, general acceptance criteria for PCDD/PCDF analyses in feed and food samples for the control of maximum levels (whose introduction in the EU was discussed at that time) were developed as contribution to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluation of PCDD/PCDF and dioxin-like PCB (Canady et al. 2002). These included quality criteria for methods applying GC/MS determination (Malisch et al. 2001) as well as bioassays (Behnisch et al. 2001) and became the basis of the EU regulations of 2002 for the control of food (European Commission 2002a) and feed (European Commission 2002b).

3.2 Limits of Quantification

The limit of detection (LOD) and/or limit of quantification (LOQ) are important parameters for the evaluation of the reliability of analytical results. For the determination of PCDD/PCDF and PCB in food, the European Commission has provided a definition and specific requirements for the LOQ (European Commission 2004), as follows:

The accepted specific LOQ of an individual congener is the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions, to be monitored with an S/N (signal/noise) ratio of 3:1 for the less sensitive signal and fulfillment of the basic requirements such as, e.g., retention time, isotope ratio according to the determination procedures as described in EPA method 1613 revision B.

This definition of the LOQ for the individual congeners was slightly modified in 2012 (European Commission 2012a; European Commission 2012b) and is one of the two pillars of the presently valid analytical criteria in the EU legislation for determination of PCDD/PCDF and dioxin-like PCB in food (European Commission 2017a) and feed (European Commission 2017b). The regulations now also allow the use of the lowest point on the calibration curve under defined conditions as alternative method for determining the LOQ. These regulations refer to the "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (EU Reference Laboratories for contaminants 2016), which provides practical advice for laboratories carrying out these determinations.

In the human milk studies described here, these conditions for the estimation of LOQ were followed for determination of all 17 PCDD/PCDF congeners with 2,3,7,8-substitution, as well as the twelve dioxin-like PCB congeners which are collectively required for the calculation of WHO-TEQ.

3.3 Upper-bound and Lower-bound Results for WHO-TEQ and ΣPCB_6 and Acceptable Differences

For calculation of the WHO-TEQ value, the results of each of the relevant congeners are multiplied by the specific TEF and then summed. In most cases, it is normal for the concentrations of a few congeners to fall below the LOQ. However, the interpretation of the results may be affected if many congeners are not quantifiable, in particular, those congeners with higher TEF values.

In order to facilitate the interpretation of the data, different imputation approaches for handling "non-detects" (more exactly: not quantified congeners) were tested using the limit of detection, among them: (i) calculation of the contribution of each non-detected congener to the TEQ as zero (lower-bound concentrations); (ii) calculation of the contribution of each non-detected congener to the TEQ as the limit of detection (upper-bound concentrations); (iii) calculation of the contribution of each non-detected congener to the TEQ as half of the limit of detection (Hoogerbrugge and Liem 2000). Later, these proposed definitions of lower- and upper-bound concentrations were used as pillars of the analytical criteria, but they were based on the LOQ rather than the detection limit.

These distinctions (upper and lower bounds) have important implications for the interpretation of the analytical results. If the contribution of non-detected congeners to the TEQ is calculated as "0", the resulting lower-bound TEQ concentrations could be interpreted as the detection of low levels of PCDD/PCDF and dioxin-like PCB in samples, whereas in effect the low TEQ content would really be the result of inadequate (not low enough) limits of quantitation. Particularly in the following cases, the measured analyte concentrations could lie near or below the limit of quantification: (i) low concentration ranges, (ii) use of mass spectrometers with insufficient sensitivity (low resolution mass spectrometers), (iii) limited sample amount available for analysis, in particular for biological samples with low lipid

content, (iv) low sample aliquot weight (for quick and easy analyses). It was therefore proposed that for food analysis, the difference between upper- and lower-bound TEQ should be in the range of 10 to 20% in defined cases (Malisch et al. 2001).

These harmonized quality criteria were included in the safety evaluation of PCDD/PCDF and dioxin-like PCB by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Canady et al. 2002) and became basis of the EU regulations for feed and food (for food as an example, see the 2002 regulation [European Commission 2002a] and their amendments until 2012 [European Commission 2012a, European Commission 2012b]). According to these requirements, the difference between the upper-bound and lower-bound WHO-TEQ level shall not exceed 20% for foodstuffs with a contamination of about 1 pg WHO-TEQ/g fat (based on the sum of PCDD/PCDF and dioxin-like PCB). Comparable requirements were laid down for the determination of PCDD, PCDF, and dioxin-like PCB in feed as well, however on a product basis (at 12% moisture). The current regulations demand that the difference between upper- and lower-bound levels shall not exceed 20% for confirmation of the exceedance of maximum levels (European Commission 2017a; European Commission 2017b). The same requirement (maximum difference below 20%) was also set as one of the criteria for the sum of NDL-PCB at the maximum level, determined by Isotope Dilution Mass Spectrometry as well as by other techniques (European Commission 2017a; European Commission 2017b).

The acceptable difference between upper- and lower-bound values is also of particular importance for the analysis of samples that are used to derive time trends in contaminant concentration, as an evaluation of the effectiveness of the Stockholm Convention: Differences that are too wide might actually be caused by inadequate analytical sensitivity and not by changes in the real levels of PCDD/PCDF and dioxin-like PCB in the samples. Therefore, a similar request was included in the guidance on the Global Monitoring Plan for persistent organic pollutants (UNEP 2013 and later amendments, including the most recent version UNEP 2019) when the following reporting format is recommended (*cit.*): "The upper-bound (ND=LOQ) and the lower-bound (ND=0) values should be given. As a QA/QC measure, the difference between these two should be less than 20%". As comment, "ND" (not detected) would be better replaced by "not quantified".

3.4 Amendments of EU Regulations; EU Guidance Documents

Various amendments were developed by the network of the EU Reference Laboratory and National Reference Laboratories for dioxins and PCB for food (European Commission 2017a) and feed (European Commission 2009) in order to improve the interpretation of the criteria. Additionally, for data obtained using confirmatory methods the regulations require that the expanded measurement uncertainty should be taken into account as described in the "Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/PCDF and PCB Analysis using Isotope Dilution Mass Spectrometry" (Eppe et al. 2017) and that the limit of

quantification should be estimated as described in the "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (EU Reference Laboratories for contaminants 2016).

3.5 Global Monitoring Plan

To provide reliable monitoring information for the Parties to the Stockholm Convention, the guidance document for the Global Monitoring Plan (GMP) proposed that a quantified objective for temporal studies should be stated, e.g. "to detect a 50% decrease in the levels of POPs within a 10 year period" (UNEP 2013; UNEP 2019). The statistical model used in the Bi-ennial Global Interlaboratory Assessment on Persistent Organic Pollutants is based on a target error of 25% as the decisive criterion to assess the performance of each laboratory for each analyte in each matrix (UNEP 2017).

4 Quality Control

Human milk samples were received at the reference laboratory over 20 years between 2000 and 2019, in five rounds, each covering approximately four years. Therefore, a rigid quality control programme was run to ensure that any differences in measured concentrations over this period did not arise from analytical variations. This approach was already applied during the third round of WHO-coordinated exposure studies (2000–2003) when the first sample was sent by one country (New Zealand) in 2000 and the last sample was received in 2003 (USA), and was continuously used in the following rounds, as well.

Accuracy depends on systematic errors and random components. "Trueness" (Closeness of agreement between the expectation of a test result or a measurement result and a true value) (ISO 3534-2: 2006, /24/) and "Precision" (closeness of agreement between independent test/measurement results obtained under stipulated conditions) (ISO 3534-2: 2006, /24/) are used to describe accuracy and are therefore important criteria for assessment of reliability of analytical methods (Eppe et al. 2017).

The comprehensive quality control programme included procedural blank samples, various kinds of in-house reference material (vegetable oil samples spiked at different levels and different kinds of quality control samples), and confirmation of certain results by duplicate analysis. Possible systematic errors were checked by the analysis of reference material or participation in numerous interlaboratory studies. This validation should guarantee a very high degree of accuracy and is part of the general quality control programme applied in the daily routine for analysis of all kinds of samples. Therefore, comprehensive validation data are available, showing the accuracy for WHO/UNEP human milk samples relative to the accuracy achieved in general routine analysis. As a result, the validation of the results gives a complex picture. The statistical evaluation of these pillars of the quality control

reflects a "worst case scenario": Analyses were performed by different operators using different chemicals over a long period (more than 20 years) with data collected in separate runs—therefore, these quality control data collected under intermediate conditions are much more robust than data from a single validation when one technician performs repeated analyses under the same conditions using the same chemicals in one sequence. The approach and detailed results for the first years were presented earlier (Malisch and van Leeuwen 2002).

The concentrations of the relevant congeners (17 PCDD/PCDF, 12 dioxin-like PCB, 6 non-dioxin-like PCB) and four main summarizing parameters (TEQ for PCDD/PCDF, dioxin-like PCB and total TEQ; ΣPCB_6) were determined with a rigid quality control as described in the next sections. 19 additional PCB congeners, which are not included in regulatory listings and therefore cannot be validated externally as part of any proficiency tests, were included in the routine analysis for a broader picture of the PCB spectrum.

4.1 Procedural Blank Samples

For PCDD/PCDF, the median of 434 procedural blank samples analysed between 2000 and 2019 is 0.04 pg WHO-PCDD/PCDF-TEQ/g (upper-bound LOQ, estimated on a lipid basis, using an aliquot equivalent to 3 g lipid for determination, see Sect. 2.1 "Analytical procedure and analytes") (Table 1). In most cases, tetra- through hexa-substituted congeners were below the LOQ. Therefore, the upper-bound calculated procedural blank is more an indication of the LOQ than a reagent blank which could be considered for possible subtraction. For dioxin-like PCB, the median value of 401 reagent blank samples was 0.01 pg WHO-PCB-TEQ/g lipid (upper-bound), and 0.05 ng/g lipid for the sum of the 6 NDL-PCB (ΣPCB_6). The median of these 434 procedural blank samples was about two orders of magnitude below the median of the 232 pooled human milk samples analysed for the WHO/UNEP-coordinated exposure studies. For samples of these studies, the maximum of procedural blank samples run in sequences together with the human milk samples had to be about one order of magnitude below the concentration of these samples. Therefore, the influence of procedural blank samples was negligible for human milk samples.

4.2 Freeze Drying and Particular PCB-related Aspects

PCB 47, PCB 51, and PCB 68 were identified as a major non-Aroclor source in residential homes. It was hypothesized that these congeners were inadvertent byproducts of polymer sealant manufacturing for finished cabinetry and produced from the decomposition of 2,4-dichlorobenzoyl peroxide used as an initiator in free-radical polymerization of polyester resins (Herkert et al. 2018).

These congeners belong neither to the group of the six indicator PCB nor to the twelve non-dioxin-like PCB and are therefore no relevant parameters for the WHO/

Table 1 WHO-PCDD/PCDF-TEQ (pg/g lipid), WHO-PCB-TEQ (pg/g lipid), and ΣPCB_6 (ng/g lipid) levels of reagent blank samples analysed together with human milk and fatty food samples between 2000 and 2019 (TEQ results with use of WHO_{1998}-TEF or WHO_{2005}-TEF; all results as upper-bound concentrations)

	WHO-PCDD/PCDF-TEQ (1998, ub) pg/g	WHO-PCDD/PCDF-TEQ (2005, ub) pg/g	WHO-PCB-TEQ (1998 ub) pg/g	WHO-PCB-TEQ (2005 ub) pg/g	ΣPCB_6 (ub) ng/g
Number of analyses	434	184	401	187	187
Median	0.04	0.04	0.01	0.01	0.05
Mean	0.06	0.05	0.06	0.02	0.15
25%-percentile	0.02	0.03	0.01	0.01	0.02
75%-percentile	0.07	0.06	0.03	0.02	0.14
90%-percentile	0.14	0.10	0.17	0.05	0.33
95%-percentile	0.19	0.13	0.31	0.07	0.56
Maximum	0.60	0.45	1.28	0.37	1.92
For comparison: Concentrations in human milk (232 samples)					
Minimum	1.21	1.01	0.27	0.27	0.90
Median	5.37	4.65	3.75	2.57	31.72

UNEP-coordinated exposure studies. However, when also other PCB congeners were included in the applied analytical method for a more complete picture, the findings of elevated PCB 47 concentrations after freeze-drying could be explained by the hypothesis of formation from 2,4-dichlorobenzoyl peroxide at production of sealants. Tests of freeze-drying of cow's milk with three different kinds of cables used for a freeze-dryer (Beta 1–8, Martin Christ) hint at the insulation of cables as the source for the PCB 47 concentrations exceeding the ranges of PCB 138, PCB 153, and PCB 180 as usually highest PCB congeners in food of animal origin and human milk (Table 2). If a method required also the determination of PCB 47, such a possible contamination could be avoided, e.g., by liquid/liquid distribution or use of adsorbents at the extraction of lipids.

The obvious contamination of submitted samples in particular with lower chlorinated PCB was detected in cases of two countries submitting samples freeze-dried instead of deep-frozen, as requested by the protocols. Seven samples from Egypt of 2001 were freeze-dried before shipment, and were apparently contaminated with lower chlorinated PCB during freeze-drying: Concentrations of the indicator-PCB PCB 28, PCB 52, PCB 101 and of the non-ortho PCB 77 and PCB 81 were an order of magnitude or more higher than usual. Hence, two additional pooled samples were submitted by Egypt in 2002, which were shipped frozen and not freeze-dried and showed the normal PCB pattern (Table 3). Furthermore, the sample from Cuba (2011) was freeze-dried before shipment. Also here, a contamination with lower

Table 2 Variation of PCB 47 concentrations in comparison to PCB 138, PCB 153, and PCB 180 in freeze-dried whole milk using different types of cables in the freeze-dryer (ng/g lipid)

Sample	Cables	PCB 47	PCB 138	PCB 153	PCB 180
Whole milk_1 a	"old" cables as used before exchange	8.42	1.43	1.96	0.80
Whole milk_1 b	New PTFE-coated cables for the rack	4.76	1.79	2.42	0.58
Whole milk_1 c	Completely new PTFE-coated cables	2.49	1.57	2.11	0.64
Whole milk_2 a	"Old" cables as used before exchange	4.03	0.94	1.29	0.64
Whole milk_2 b	New PTFE-coated cables for the rack	2.40	1.35	1.92	0.51
Whole milk_2 c	Completely new PTFE-coated cables	1.04	1.30	1.79	0.54
Whole milk_3 a	"Old" cables as used before exchange	7.25	1.17	1.57	0.61
Whole milk_3 b	New PTFE-coated cables for the rack	3.32	1.21	1.71	0.43
Whole milk_3 c	Completely new PTFE-coated cables	1.64	1.07	1.49	0.48

chlorinated PCB occurred during freeze-drying and resulted in an unusual PCB pattern (Malisch et al. 2023b). It is unknown what exactly might have caused this contamination in these submitted samples, e.g. sealants or pump oil, as freeze-drying with suitable instruments is a well-proven contamination-free technique. Anyways, samples have to be shipped deep-frozen or, in cases when frozen receipt by the reference laboratory cannot be guaranteed, after addition of a small amount of potassium dichromate ($K_2Cr_2O_7$) to the sample for stabilization (Malisch et al. 2023a).

4.3 Vegetable Oil Samples Fortified at Different Levels as in-house Reference Material

Fortification of refined vegetable oil (sunflower oil) with different levels of native PCDD/PCDF and PCB is a well-established procedure to check the recovery of native analytes and variation at various levels. Therefore, starting with the analysis of the human milk samples for the "third round" performed between 2000 and 2003, the fortification experiments for control of the usual contamination of food and feed were expanded to also over the higher levels that are found in human milk in order to check the linearity of the response for PCDD/PCDF (range of about 0.6 to 25 pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid and 1 to 40 pg WHO-PCB-TEQ_{1998}/g lipid). Table 4 summarizes the recoveries and relative standard deviation (RSD) of fortification tests performed between 1994 and 2003 for WHO-PCDD/PCDF-TEQ_{1998}.

Table 3 PCB concentrations in pooled human milk samples from Egypt (pools 1–7 freeze-dried before shipment in 2001; pools 8–9 sent deep-frozen in 2002; marked in italics: extremely high concentrations of PCB 28, PCB 52, PCB 101, PCB 77, and PCB 81 caused by freeze-drying before shipment; ng/g lipid for the six indicator PCB [PCB 28–PCB 180] and the 8 mono-ortho dioxin-like PCB [PCB 105–PCB 189]; pg/g lipid for the four non-ortho dioxin-like PCB [PCB 77–PCB 169])

	Freeze-dried before shipment							Deep-frozen shipment	
	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6	Pool 7	Pool 8	Pool 9
	Rural	Sub-urban	Urban	Sub-urban	Rural/ industrial	Rural/ upper Egypt	Sub-urban/ upper Egypt	Rural/ upper Egypt	Rural/ upper Egypt
Lipid (%)	20.2	31.5	26.3	28.4	27.9	26.4	19.7	3.1	3.1
PCB 28	*63.75*	*73.34*	*58.00*	*74.42*	*72.23*	*57.60*	*65.41*	2.89	1.14
PCB 52	*16.58*	*18.70*	*11.45*	*18.47*	*16.00*	*10.72*	*12.26*	0.30	0.41
PCB 101	*5.72*	*7.82*	*3.64*	*5.63*	*4.92*	*2.47*	*3.00*	0.41	0.41
PCB 138	11.54	11.69	8.49	8.09	15.71	10.93	8.98	6.05	3.26
PCB 153	11.54	12.49	8.91	8.72	18.71	12.52	9.76	6.49	3.89
PCB 180	7.12	8.42	6.30	5.49	12.63	8.21	6.48	4.19	2.95
PCB 105	1.58	3.38	1.20	1.57	2.34	1.23	1.46	0.88	0.44
PCB 114	< 0.54	< 0.25	< 0.30	< 0.28	< 0.18	< 0.31	< 0.28	0.10	0.06
PCB 118	4.39	6.72	3.06	3.92	5.89	3.99	3.94	2.46	1.34
PCB 123	< 0.52	< 0.24	< 0.30	< 0.28	< 0.18	< 0.31	< 0.27	0.07	0.05
PCB 156	1.26	1.34	0.89	0.83	1.94	1.35	0.92	0.66	0.40
PCB 157	0.25	0.28	0.19	0.18	0.37	0.37	0.21	0.15	0.10
PCB 167	0.62	0.55	0.42	0.37	0.73	0.75	0.53	0.36	0.23
PCB 189	0.15	0.14	0.12	0.09	0.19	0.20	0.11	0.08	0.06
PCB 77	*675.2*	*672.2*	*373.6*	*627.8*	*416.3*	*244.7*	*239.6*	4.7	6.0
PCB 81	*47.5*	*138.2*	*28.7*	*59.6*	*36.5*	*22.9*	*27.0*	6.9	4.4
PCB 126	40.2	47.4	30.7	37.0	56.5	61.9	39.8	43.9	38.1
PCB 169	22.7	19.9	15.4	14.8	23.1	44.7	19.0	13.5	13.5

Table 4 Mean recovery and RSD (%) of samples fortified at various WHO-PCDD/PCDF-TEQ_{1998} concentrations (pg/g lipid) analysed together with human milk and food samples between 1994 and 2003

Fortified level pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid	No of replicates	Mean (pg/g lipid)	Mean recovery (%)	RSD (%)
0.61	8	0.62	103	5.4
1.21	87	1.23	101	7.1
2.43	40	2.48	102	6.9
6.07	3	5.64	93.0	1.8
9.71	5	9.91	102	8.9
24.3	1	24.6	101	–

Table 5 Mean recovery and RSD (%) of samples fortified at various WHO-PCB-TEQ_{1998} concentrations (pg/g lipid) analysed together with human milk samples between 2000 and 2003

Fortified level pg WHO-PCB-TEQ_{1998}/g lipid	No of replicates	Mean (pg/g lipid)	Mean recovery (%)	RSD (%)
7.99	4	8.40	105	12
15.5	1	15.2	97.8	–
32.8	3	29.6	91.4	1.7
39.9	10	37.9	95.0	2.2

The mean recoveries at six fortification levels (range 0.6–24.3 pg/g) were in the range between 93 and 103% with an RSD between 2 and 9%.

Table 5 summarizes the results for WHO-PCB-TEQ_{1998} of fortified samples which were performed between 2000 and 2003 in combination of analyses of human milk samples of the third round (2000–2003). The mean recoveries at 4 fortification levels were between 91 and 105% with an RSD between 1.7 and 11.5%.

As result, the quality parameters are comparable at different fortification levels including the lower concentrations that were usually found in food and the higher concentrations found in human milk.

4.4 Quality Control Samples as in-house Reference Material and Precision

Numerous quality control samples have been used for monitoring of the precision of PCDD/PCDF analysis since 1994. Initially, samples of butter and extracted lipids from eggs that were contaminated at different levels were used. Table 6 summarizes the results obtained for these samples between 1994 and 2003 as indication of the variation of results (expressed as RSD [%]) at times of the analyses of human milk during the 2000–2003 round.

Figure 1 illustrates the quality control chart for butter A over the whole period of its use from 1994 until 2007 (thus, including the third and fourth round performed

Table 6 RSD (%) of quality control samples in the range between about 0.7 pg/g and 5 pg/g WHO-PCDD/PCDF-TEQ_{1998} used between 1994 and the end of the 2000–2003 round

Sample	Mean level (pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid)	No of replicates	RSD (%)
Butter A	0.67	62	8.7
Butter B	1.36	51	7.4
Egg fat A	0.80	47	10.5
Egg fat B	4.95	25	8.7

between 2000 and 2007). In this time, 100 replicates were analysed by numerous technicians under varying conditions—various batches of chemicals, instrumental conditions, etc. Around the mean (M), warning levels are set at two sigma (lower warning level at M-2 s, upper warning level at M + 2 s), control levels at three sigma (lower control level at M-3 s, upper control level at M + 3 s). In the 13 years of use of this quality control sample, an RSD of nearly 10% was observed for a mean level of 0.67 pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid. The lower warning level of 0.54 pg/g was reached by one of the 100 replicates, the upper warning level of 0.80 pg/g exceeded by three samples. None of the 100 replicates exceeded the lower (0.44 pg/g) or upper (0.90 pg/g) control level which would have required a thorough follow-up to identify the reason for the deviation. Note that butter A with a concentration of 0.67 pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid was on average about a factor of 10 lower than the concentrations found in human milk between 2000 and 2003 (range 3.3–22.3 pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid) and therefore demonstrates the performance even at the much lower concentration ranges that were found in many food samples of animal origin (cow's milk, poultry, beef, veal, hen's eggs) at that time (EU Scientific Committee on Food 2000; EFSA 2012).

Over time, the number of quality control samples was expanded considerably: 10 different quality control samples (mixed fat, milk fat, egg fat, fish oil, and pork fat) were used between 2003 and 2019 for control of food and human milk samples (see Table 7) covering a range between about 2 and 12 pg WHO-PCDD/F-PCB-TEQ_{2005}/g lipid, and 7 to 80 ng/g ΣPCB_6. For all samples and all sum parameters, the RSD was between 3 and 11% (median about 7 to 8%).

In conclusion, based on the clean-up and GC-HRMS determination of these quality control samples, the methodology achieved a long-term precision of below 15% over the 2000–2019 period.

4.5 Duplicate Analysis and Precision

PCDD/PCDF and PCB concentrations of most samples were determined by duplicate analyses. At the study performed between 2000 and 2003, this approach was optimized in a way which can best be described as the "overlapping sandwich method". A large portion of the samples was analysed in duplicate with the second confirmatory analysis being performed in sequences with samples from other

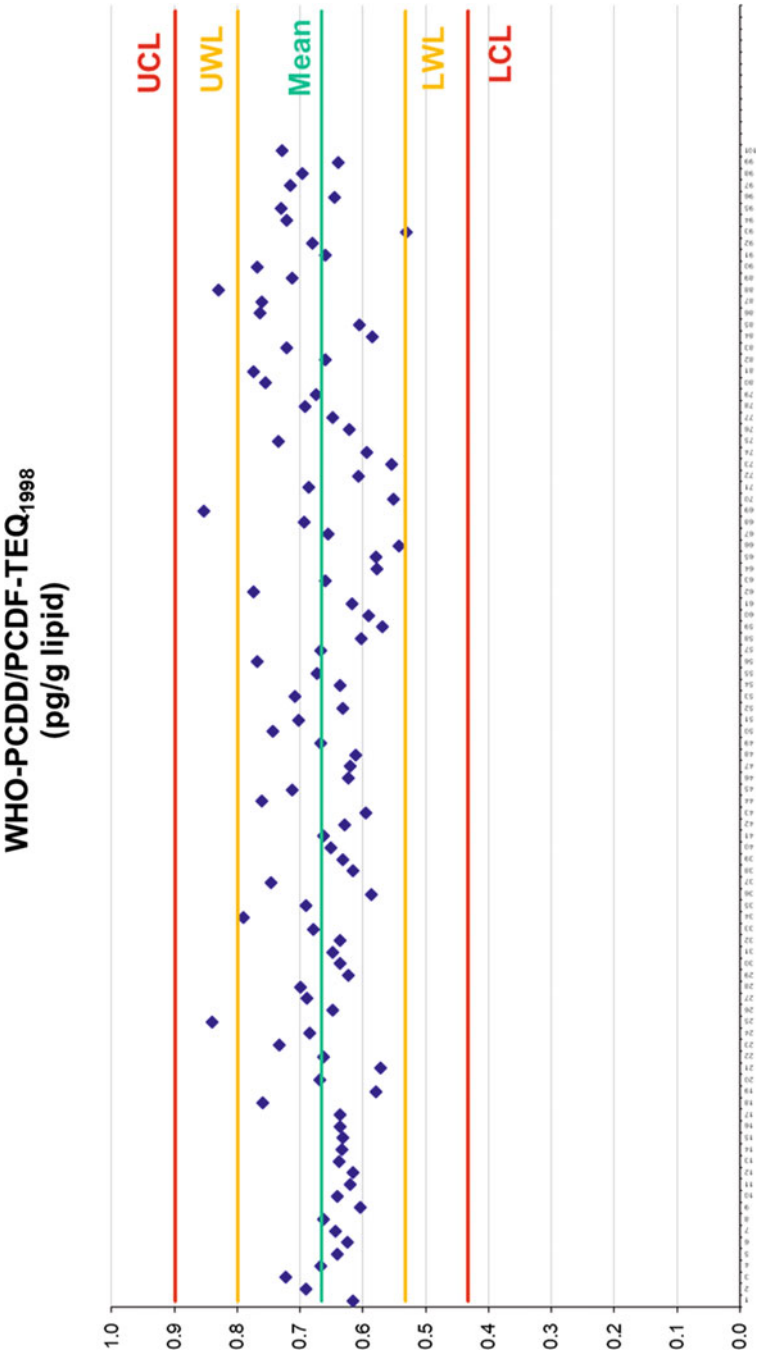

Fig. 1 Quality control chart for "butter A" (0.67 pg WHO-PCDD/PCDF-TEQ_{1998}/g lipid) used from 1994 to 2007 (UCL = upper control level, UWL = upper warning level, LWL = lower warning level, LCL = lower control level)

Table 7 Quality control samples used between 2003 and 2019

No	Sample	Used		no of replicates	WHO-PCDD/ PCDF-PCB-TEQ (2005)	WHO-PCDD/ PCDF-TEQ (2005)	WHO-PCB-TEQ (2005)	ΣPCB_6	WHO-PCDD/ PCDF-TEQ (2005)	WHO-PCB-TEQ (2005)	ΣPCB_6
		from	until		pg/g lipid	pg/g lipid	pg/g lipid	ng/g lipid	RSD (%)	RSD (%)	RSD (%)
1	Mixed fat—1	2015	2019	102	2.0	0.9	1.2	25.0	10	8	9
2	Mixed fat—2	2003	2018	443	2.4	0.8	1.5	11.9	10	10	11
3	Milk fat—1	2007	2013	115	2.6	0.7	1.9	8.8	7	7	3
4	Milk fat—2	2013	2019	114	2.9	1.8	1.1	20.5	8	8	7
5	Egg fat—1	2015	2019	71	1.9	1.2	0.7	6.5	7	9	7
6	Egg fat—2	2005	2015	82	8.8	3.4	5.5	70.8	8	8	10
7	Fish oil—1	2007	2019	135	11.9	1.9	10.1	80.2	8	7	5
8	Fish oil—2	2008	2019	68	7.2	1.0	6.2	59.7	8	6	4
9	Pork fat—1	2010	2019	55	3.2	0.7	2.5	15.4	7	7	5
10	Pork fat—2	2013	2019	145	2.4	1.2	1.1	30.7	7	7	7
	Min			55	1.9	0.7	0.7	6.5	7	6	3
	Median			108	2.8	1.1	1.7	22.8	8	8	7
	Max			443	11.9	3.4	10.1	80.2	10	10	11

Table 8 RSD (%) for determination of WHO-PCDD/PCDF-TEQ_{1998} based on duplicate analyses of 74 human milk samples received between 2000 and 2003 with differentiation into five concentration ranges

	WHO-PCDD/PCDF-TEQ (WHO_{1998}-TEF; pg/g lipid)				
Range	< 5	5–8	8–11	11–15	> 15
No. of samples	8	24	23	11	8
Mean	3.82	6.59	9.40	12.8	18.1
RSD (%)	4.9	2.7	2.3	3.6	5.8

Table 9 RSD (%) of determination of WHO-PCB-TEQ_{1998} based on duplicate analyses of 74 human milk samples received between 2000 and 2003

	WHO-PCB-TEQ pg/g lipid	WHO-PCB-TEQ pg/g lipid	WHO-PCB-TEQ pg/g lipid	WHO-PCB-TEQ pg/g lipid
Range	< 5	5–10	10–17	> 17
No of samples	28	22	17	7
Mean of all samples	3.56	6.98	13.2	21.5
RSD (%)	7.3	4.6	3.4	4.2

countries and with different quality control samples. This ensured that the results of all samples from different countries had the same reliability despite receipt of the various samples by the reference laboratory over a period of three years: The combination of the "overlapping sandwich method" and use of quality control samples ensured that the sample results in this round of the study of the first country sending samples (New Zealand, 2000) had the same reliability as the last country (USA, 2003). Any differences in levels could therefore be attributed to the result of real concentration differences and not of any analytical variation. In addition, it is possible to calculate the repeatability standard deviation from duplicate analyses.

As an example, from 102 samples received from 26 countries between 2000 and 2003, 64 were analysed as duplicates and additionally, 10 were analysed as triplicates. These 74 samples were sorted into five groups with different ranges of WHO-PCDD/PCDF-TEQ_{1998} concentrations. The repeatability standard deviation (RSD) was calculated using the sum of differences of the individual results to the mean results of the samples. Table 8 summarizes the results for the RSD (%) for the mean concentration in each range. At all levels (from <5 pg WHO-PCDD/PCDF-TEQ/g lipid to >15 pg WHO-PCDD/PCDF-TEQ/g lipid), the RSD was between 2 and 6%.

Table 9 summarizes the results for the calculation of RSD (%) for the 74 samples analysed as duplicates or triplicates for WHO-PCB-TEQ_{1998} with differentiation into 4 groups (from "<5 pg WHO-PCB-TEQ/g lipid" to ">17 pg WHO-PCB-TEQ/g lipid"). The RSD of the mean WHO-PCB-TEQ concentrations in the respective groups was in the range between 3 and 7%. Furthermore, in Table 10, also the mean of the corresponding NDL-PCB results in these 4 groups is given (range between

Table 10 RSD (%) of determination the sum of the 6 non-dioxin-like PCB (ΣPCB_6) based on duplicate analyses of 74 human milk samples received between 2000 and 2003

	WHO-PCB-TEQ pg/g lipid	WHO-PCB-TEQ pg/g lipid	WHO-PCB-TEQ pg/g lipid	WHO-PCB-TEQ pg/g lipid
Range	< 5	5–10	10–17	> 17
No of samples	28	22	17	7
Results for the corresponding NDL-PCB concentrations (ng NDL-PCB/g lipid)				
Mean of all samples	34.8	120	184	431
RSD (%)	3.1	2.4	1.5	1.9

35 and 430 ng ΣPCB_6/g lipid); the RSD for the NDL-PCB determinations was between 2 and 3%.

4.6 Pooled Human Milk Samples Remaining from WHO Interlaboratory Assessment Study 1995–1996 as Quality Control Samples 2000–2003

At the fourth quality assessment study conducted by WHO on levels of PCB, PCDD, and PCDF in human milk in 1995 and 1996 (WHO 2000), the CVUA Freiburg was designated as the WHO Reference Laboratory for the following WHO exposure study. In order to check the analytical reliability for the WHO-coordinated exposure study 2000–2003, four pools of human milk samples remaining from the 1995–1996 WHO interlaboratory assessment study were provided to CVUA Freiburg for inclusion as quality control samples. This allowed a check on whether the performance of the quality assessment study could be reproduced about six years later when various human milk samples from different countries were analysed. Three of the four samples had a sufficient sample amount allowing repeated use in two different analytical sequences. Thus, seven replicates could be performed between 2000 and 2003, when samples from different countries were analysed. These quality control data were obtained under intermediate conditions by analyses of different operators using different chemicals over a long period in separate runs.

A main criterion for the evaluation of the fourth quality assessment study conducted by WHO was the long-time reliability of the analytical performance. Trueness and precision of measurements was assessed for compounds classified as group I (the most important congeners with dioxin-like properties: 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, PCB 126, PCB 118, PCB 156, PCB 157) and group II (the most abundant non-dioxin-like PCB: PCB 138, PCB 153, PCB 180).

Table 11 summarizes the mean recoveries of the measurements for the seven replicates of remaining human milk samples between 2000 and 2003 in comparison to the results submitted for the 1995–1996 quality WHO assessment study and the corresponding RSDs (with split of WHO-PCB-TEQ into two subgroups of

Table 11 Mean recoveries (%) and RSDs (%) of seven replicates of remaining human milk of samples used as quality control samples between 2000 and 2003 in comparison to the results submitted for the 1995–1996 quality WHO assessment study

	Mean recoveries (%) 2000–2003 in comparison to 1995–1996	RSD (%)
WHO-PCDD/PCDF-TEQ$_{1998}$	101	7.1
Non-ortho-PCB-TEQ$_{1988}$	83.9	13.4
Mono-ortho-PCB-TEQ$_{1998}$	92.3	10.7
ΣPCB_6	96.4	11.2
Lipid content	92.8	4.1

non-ortho PCB and mono-ortho PCB). The RSDs for the decisive individual congeners were for PCDD/PCDF (2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF) in the range 7 to 12%, for PCB 126 14%, for mono-ortho PCBs (PCB 118, PCB 156, PCB 157) in the range 10–14%, and for non-dioxin-like PCB (PCB 138, PCB 153, PCB 180) 10–11%. As a result, these measurements between 2000 and 2003 were in line with the 1995–1996 submitted results, when the criteria for acceptance of the fourth quality assessment study were met.

4.7 Participation in Proficiency Tests and Trueness

Between 2000 and 2019, the CVUA Freiburg successfully participated in 32 proficiency tests covering 81 test samples of food of animal origin or human milk (Table 17 in the appendix).

In order to summarize the evaluation of all 81 samples of these proficiency tests performed between 2000 and 2019, the results for 52 samples were calculated on a lipid basis, 29 on product basis (based on the specifications of the EU regulations for maximum levels for WHO-PCDD/PCDF-PCB-TEQ and for ΣPCB_6), regardless of the requirements for reporting data for individual proficiency tests. Figure 2 depicts the deviation (%) of results of CVUA Freiburg for WHO-PCDD/PCDF-PCB-TEQ from the consensus values, Fig. 3 for WHO-PCDD/PCDF-TEQ, Fig. 4 for WHO-PCB-TEQ, and Fig. 5 for the sum of the 6 NDL-PCB. In all figures, the deviation for results calculated on a lipid and whole basis is marked as blue and red squares, respectively. Green dotted lines mark the lower and upper end of the range relevant for human milk samples (minimum and maximum found in all 232 submitted pooled samples). There was no differentiation between TEQ results based on the use of WHO-TEF$_{1998}$ or WHO-TEF$_{2005}$.The general idea of this summarizing evaluation was to check how the results reported by CVUA Freiburg deviated from the respective consensus value—and this deviation has always been calculated on the same basis (same TEF, same basis "lipid" or "product").

As result, in the range relevant for human milk above 1 pg WHO-TEQ/g lipid, about 90% of the results differed by less than 10% from the assigned values for

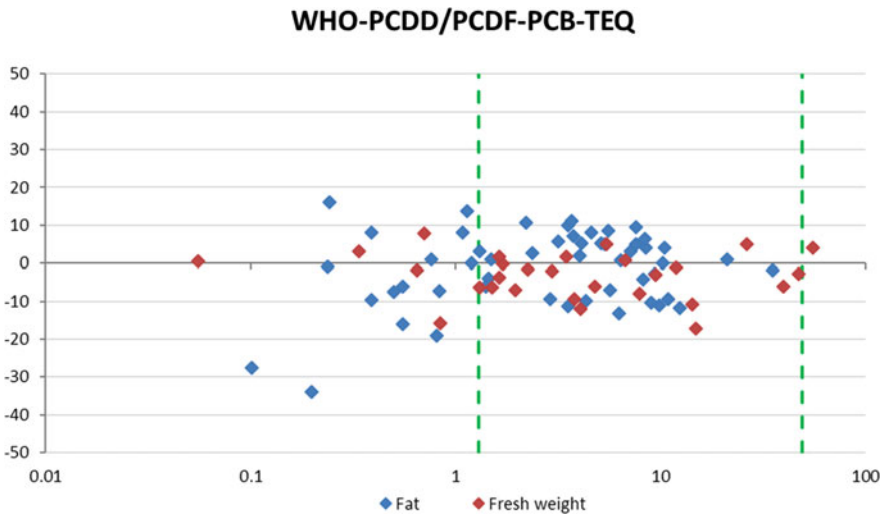

Fig. 2 Deviation (%) of results of CVUA Freiburg for WHO-PCDD/PCDF-PCB-TEQ from the assigned values of proficiency tests performed between 2000 and 2019 (blue squares: results on lipid base as pg WHO-PCDD/PCDF-PCB-TEQ/g lipid; red squares: results on product base as pg WHO-PCDD/PCDF-PCB-TEQ/g product; green dotted line: range relevant for human milk samples [about 1–50 pg/g lipid])

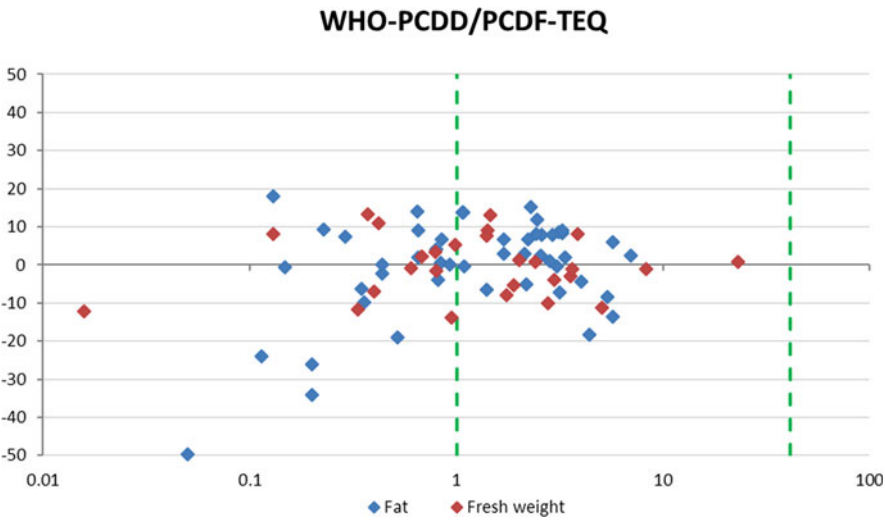

Fig. 3 Deviation (%) of results of CVUA Freiburg for WHO-PCDD/PCDF-TEQ from the assigned values of proficiency tests performed between 2000 and 2019 (blue squares: results on lipid base as pg WHO-PCDD/PCDF-TEQ/g lipid; red squares: results on product base as pg WHO-PCDD/PCDF-TEQ/g product; green dotted line: range relevant for human milk samples [about 1–40 pg/g lipid])

Fig. 4 Deviation (%) of results of CVUA Freiburg for WHO-PCB-TEQ from the assigned values of proficiency tests performed between 2000 and 2019 (blue squares: results on lipid base as pg WHO-PCB-TEQ/g lipid; red squares: results on product base as pg WHO-PCB-TEQ/g product¸ green dotted line: range relevant for human milk samples [about 0.3–15 pg/g lipid])

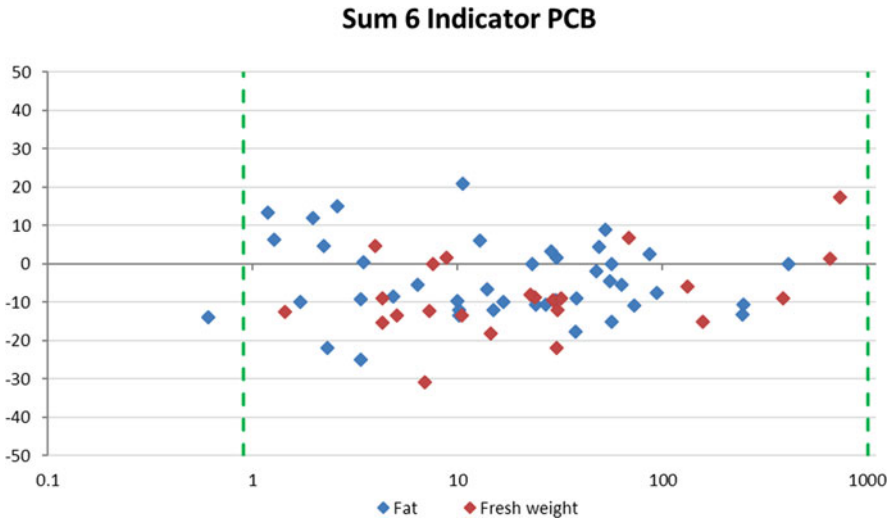

Fig. 5 Deviation (%) of results of CVUA Freiburg for the sum of the 6 NDL-PCB ($\Sigma PCB_{6)}$ from the assigned values of proficiency tests performed between 2000 and 2019 (blue squares: results on lipid base as ng ΣPCB_6 /g lipid; red squares: results on product base as ng ΣPCB_6/g product; green dotted line: range relevant for human milk samples [about 1–1000 ng/g lipid])

WHO-PCDD/PCDF-PCB-TEQ and WHO-PCDD/PCDF-TEQ, and by about 15% for WHO-PCB-TEQ. In the wider range relevant for the sum of 6 NDL-PCB (1–1000 ng/g lipid), about 90% of the results differed by less than 15% from assigned values. The maximum deviation at these relevant concentrations was less than about 15–20% for the TEQ-based parameters, and about 25% for the sum of the 6 NDL-PCB.

The observed tendency to higher deviations in some proficiency tests for the sum of the 6 NDL-PCB raises some questions about analytical performance. NDL-PCB are found at much higher concentrations than PCDD/PCDF or DL-PCB. Therefore, it is assumed that NDL-PCB analysis would be easier and provide more accurate results than PCDD/PCDF-analysis. In contrast to the observations in proficiency tests, the internal quality control data gathered over about two decades do not show a reason for such a higher deviation: The RSD of the quality control samples (see Sects. 4.3 and 4.4) and of the replicates of left-overs of the quality WHO assessment samples (see Sect. 4.6) shows no difference between TEQ-based results and the sum of the 6 NDL-PCB. The duplicate analyses of samples of these studies give even slightly better RSDs for the sum of the 6 NDL-PCB in comparison to the TEQ-based results (see Sect. 4.5). Therefore, it is assumed that the deviations in some proficiency tests might result more from higher uncertainty of the assigned values in certain tests rather than from internal analytical deficiencies. The analytical methodology for the determination of NDL-PCB at CVUA-Freiburg has always used ^{13}C-labelled internal standards and HRGC-HRMS determination (see Sect. 2.1). However, for many years, a number of laboratories have also used other techniques, often not controlling the quantification of the 6 NDL-PCBs by ^{13}C-labelled internal standards. Therefore, in some cases, the calculation of the assigned value for NDL-PCB might have been based on a wider distribution of results.

Figure 6 demonstrates the consistency of the performance over time by a plot showing the average deviation per year in % of results of CVUA Freiburg from assigned values of proficiency tests performed between 2000 and 2019 for WHO-PCDD/PCDF-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ, and sum of 6 NDL-PCB covering the respective relevant ranges.

4.8 Accreditation

In 1993, new quality standards were introduced for laboratories entrusted with the official control of foodstuffs by the Member States of the European Economic Community. Laboratories had to comply with the general criteria for the operation of testing laboratories laid down in European Standard EN 45001 supplemented by standard operating procedures and the random audit of their compliance by quality assurance personnel not later than November 1998 (Council Directive 93/99/EEC (1993)). In a revision of the regulations on official controls in 2004 (EU Regulation 882/2004), it was stipulated that laboratories that were designated for official control should operate and be assessed and accredited in accordance with the European Standard EN ISO/IEC 17025—"General requirements for the competence of testing

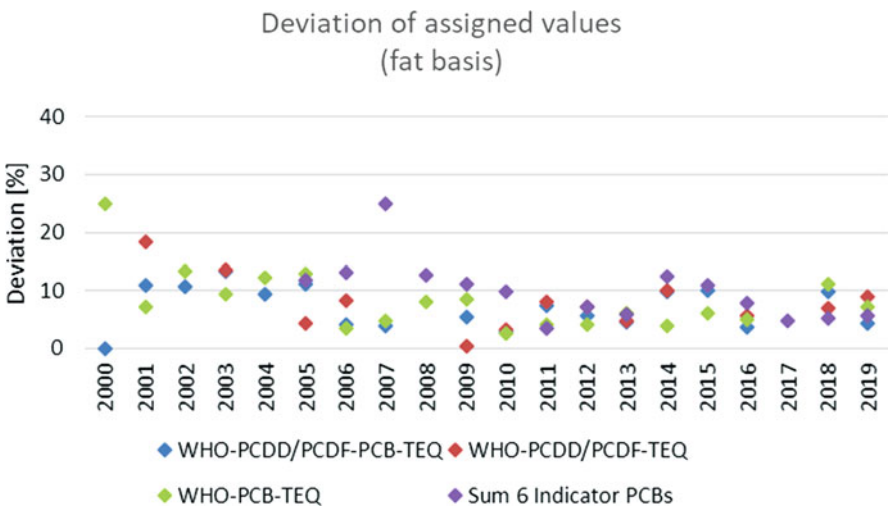

Fig. 6 Average deviation per year in % of results of CVUA Freiburg from assigned values of proficiency tests performed between 2000 and 2019 for WHO-PCDD/PCDF-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ, and sum of 6 NDL-PCB covering the respective relevant ranges

and calibration laboratories" (European Standard EN ISO/IEC 17025 (2017)). Therefore, the CVUA Freiburg was accredited in 1998 and has since been re-accredited continuously.

As a result, all analyses performed by CVUA Freiburg for the WHO/UNEP-coordinated exposure studies since 2000 followed the strict rules of the accreditation system and the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025.

5 Summary and Conclusions

In support of the WHO/UNEP-coordinated exposure studies on PCDD/PCDF and PCB in human milk, comprehensive quality control was performed on the received samples over two decades. As analytical criteria for human milk are not specified, the criteria for the analyses of PCDD/PCDF and PCB in feed and food required by EU legislation were used. The analytical methodology and validation data used were applicable for the analysis of the human milk samples and met the regulatory requirements for acceptance of results for foods including milk. Supporting this, the CVUA Freiburg participated successfully in 32 proficiency tests between 2000 and 2019, covering 81 test samples of food of animal origin or human milk, including two UNEP-interlaboratory studies on POPs.

As a key parameter supporting the reliability of these studies, trueness was estimated from the proficiency test samples in the relevant range above 1 pg

WHO-TEQ/g lipid. The deviation was less than 10% from assigned values for WHO-PCDD/PCDF-PCB-TEQ and WHO-PCDD/PCDF-TEQ and less than about 15% for WHO-PCB-TEQ, for about 90% of the results. In the wider range relevant for the sum of 6 NDL-PCB (1–1000 ng/g lipid), about 90% of the results differed by less than 15% from assigned values.

Furthermore, it has been proven that the results meet an important criterion for monitoring data on the presence of these contaminants in order to identify trends in levels. The guidance document for the Global Monitoring Plan recommends, as a quantitative objective for temporal studies, the detection of a 50% decrease in the levels of POPs within a time period of 10 years. Therefore, UNEP-coordinated interlaboratory assessments are evaluated based on a target error of 25%. A long-term fulfilment of the criterion of a variation of ±25% for the determination of PCDD/PCDF and PCB was demonstrated by participation in the 2000–2019 proficiency tests. Additionally, a long-term precision of below 15% was achieved, based on quality control samples analysed during the above-mentioned period.

Collectively, these controls ensured that any differences in concentration levels in this wide span of altogether 20 years between 2000 and 2019, e.g. lower levels found in later years, did not arise from any analytical variation but were the result of decreasing time trends.

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratory, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the Reference Laboratory was an important aspect for the successful organization of these studies on a global level.

Hae Jung Yoon, Seongsoo Park, and Philippe Verger (Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

We thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao, and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

We greatly appreciate the performance of the PCDD/PCDF and PCB analyses by the team at CVUA Freiburg. Working in the dioxin laboratory since its build-up in 1991, Renate Tritschler with her reliable work from extraction via complex clean-up steps to preparation of the purified extracts ready for MS analysis and Helmut Winterhalter as careful operator of the HRGC/HRMS systems have contributed significantly to the successful establishment of analytical methods. Furthermore, Thomas Huber, Stephanie Schwaab, Emmy Kopf, Michaela Fritz, Indra Peters, Jutta Schächtele, Cornelia Adamovic, Lena Zeug, and Kirsten Denninger are acknowledged for excellent laboratory work and Jutta Schächtele, Stefan Leswal, and Lukas Jänker for HRGC/HRMS measurements. Finally, we thank Christian Wambold, Nicole Bitomsky Kerstin Wahl, Katharina Djuchin, Sandra Schill, and Annika Maixner for their scientific support. Alwyn Fernandes, University of East Anglia, UK, is gratefully acknowledged for his editorial support.

Appendix

Table 12 Native and $^{13}C_{12}$-labelled PCDD/PCDF with 2,3,7,8-substitution

	WHO-TEF 1998	WHO-TEF 2005	native	$^{13}C_{12}$-labelled
2,3,7,8-TCDD	1	1	X	X
1,2,3,7,8-PeCDD	1	1	X	X
1,2,3,4,7,8-HxCDD	0.1	0.1	X	X
1,2,3,6,7,8-HxCDD	0.1	0.1	X	X
1,2,3,7,8,9-HxCDD	0.1	0.1	X	X
1,2,3,4,6,7,8-HpCDD	0.01	0.01	X	X
OCDD	0.0001	0.0003	X	X
2,3,7,8-TCDF	0.1	0.1	X	X
1,2,3,7,8-PeCDF	0.05	0.03	X	X
2,3,4,7,8-PeCDF	0.5	0.3	X	X
1,2,3,4,7,8-HxCDF	0.1	0.1	X	X
1,2,3,6,7,8-HxCDF	0.1	0.1	X	X
1,2,3,7,8,9-HxCDF	0.1	0.1	X	X
2,3,4,6,7,8-HxCDF	0.1	0.1	X	X
1,2,3,4,6,7,8-HpCDF	0.01	0.01	X	X
1,2,3,4,7,8,9-HpCDF	0.01	0.01	X	X
OCDF	0.0001	0.0003	X	X

Table 13 Native and $^{13}C_{12}$-labelled non-ortho PCB (dioxin-like PCB with attributed TEF factor in bold)

		WHO-TEF 1998	WHO-TEF 2005	native	$^{13}C_{12}$-labelled
PCB 37	344′-TriCB	–	–	X	X
PCB 77	**33′44′-TeCB**	0.0001	0.0001	**X**	**X**
PCB 81	**344′5-TeCB**	0.0001	0.0003	**X**	**X**
PCB 126	**33′44′5-PeCB**	0.1	0.1	**X**	**X**
PCB 169	**33′44′55′-HxCB**	0.01	0.03	**X**	**X**

Table 14 Native and $^{13}C_{12}$-labelled mono-ortho PCB (dioxin-like PCB in bold; initially available as $^{13}C_{12}$-labelled internal standards PCB 28, PCB 105, PCB 118, PCB 156, and PCB 189)

		WHO-TEF 1998	WHO-TEF 2005	native	$^{13}C_{12}$-labelled
PCB 28	244′-TriCB	–	–	X	X
PCB 33	2′34-TriCB	–	–	X	
PCB 55	233′4-TeCB	–	–	X	
PCB 60	2344′-TeCB	–	–	X	X
PCB 66	23′44′-TeCB	–	–	X	
PCB 74	244′5-TeCB	–	–	X	
PCB 105	**233′44′-PeCB**	**0.0001**	**0.00003**	**X**	**X**
PCB 110	233′4′6-PeCB	–	–	X	
PCB 114	**2344′5-PeCB**	**0.0005**	**0.00003**	**X**	**X**
PCB 118	**23′44′5-PeCB**	**0.0001**	**0.00003**	**X**	**X**
PCB 122	2′33′45-PeCB	–	–	X	
PCB 123	**2′344′5-PeCB**	**0.0001**	**0.00003**	**X**	**X**
PCB 124	2′3455′-PeCB	–	–	X	
PCB 156	**233′44′5-HxCB**	**0.0005**	**0.00003**	**X**	**X**
PCB 157	**233′44′5′-HxCB**	**0.0005**	**0.00003**	**X**	**X**
PCB 167	**23′44′55′-HxCB**	**0.00001**	**0.00003**	**X**	**X**
PCB 189	**233′44′55′-HpCB**	**0.0001**	**0.00003**	**X**	**X**

Table 15 Native and $^{13}C_{12}$-labelled di/tri/tetra-ortho PCB (the 6 indicator PCB marked in bold); *PCB 28 is a mono-ortho PCB which is one of the 6 indicator PCB

		6 indicator PCB	native	$^{13}C_{12}$-labelled
PCB 18	22′5-TriCB		X	
PCB 28*	***244′-TriCB***	**X**	**X**	**X**
PCB 52	**22′55′-TeCB**	**X**	**X**	**X**
PCB 99	22′44′5-PeCB		X	
PCB 101	**22′455′-PeCB**	**X**	**X**	**X**
PCB 128	22′33′44′-HxCB		X	
PCB 138	**22′344′5′-HxCB**	**X**	**X**	**X**
PCB 141	22′3455′-HxCB		X	X
PCB 153	**22′44′55′-HxCB**	**X**	**X**	**X**
PCB 170	22′33′44′5-HpCB		X	X
PCB 180	**22′344′55′-HpCB**	**X**	**X**	**X**
PCB 183	22′344′5′6-HpCB		X	
PCB 187	22′34′55′6-HpCB		X	
PCB 194	22′33′44′55′-OcCB		X	X
PCB 206	22′33′44′55′6-NonaCB		X	X
PCB 209	22′33′44′55′66′-DecaCB		X	X

Table 16 $^{13}C_{12}$-labelled PCDD/PCDF without 2,3,7,8-substitution used ad recovery standards (1,2,3,4-TCDD used in all studies; other $^{13}C_{12}$-labelled PCDD/PCDF without 2,3,7,8-substitution being used since 2018)

	$^{13}C_{12}$-labelled PCDD/PCDFs without 2,3,7,8-substitution
1,2,3,4-TCDD	X
1,2,3,4,7-PeCDD	X
1,2,3,4,6,8-HxCDD	X
1,2,3,4,6,7,9-HpCDD	X
1,2,7,8-TCDF	X
1,2,3,4,6-PeCDF	X
1,2,3,4,6,9-HxCDF	X
1,2,3,4,6,8,9-HpCDF	X

Table 17 Participation in proficiency test in the period 2000–2019

No	Year	Matrix	Organzier
1	2000	Chicken meat	Norwegian Institute of Public Health
		Butter	
		Fish	
2	2001	Beef meat	Norwegian Institute of Public Health
		Cod liver	
		Human milk	
3	2002	Tuna	Norwegian Institute of Public Health
		Pork meat	
		Egg yolk	
4	2003	Cheese	Norwegian Institute of Public Health
		Turkey	
		Salmon	
5	2004	Palm oil	Norwegian Institute of Public Health
		Chicken meat	
		Trout	
6	2005	Cod liver oil	Norwegian Institute of Public Health
		Reindeer	
		Herring	
7	2006	Egg yolk	Norwegian Institute of Public Health
		Halibut	
		Human milk	
8	2007	Salmon	Norwegian Institute of Public Health
		Chicken meat	
		Butter	
9	2008	Venison	Norwegian Institute of Public Health
		Eel	
		Cream	
10	2009	Beef meat	Norwegian Institute of Public Health
		Butter	
		Herring	
11	2010	Human milk	Norwegian Institute of Public Health
		Pork meat	
		Trout	
12	2010	Pork fat	EU-RL for Dioxins and PCBs in Feed and Food
		Milk fat	
13	2011	Salmon	Norwegian Institute of Public Health
		Mozzarella	
		Chicken egg	
14	2011	Salmon	EU-RL for Dioxins and PCBs in Feed and Food
		Fish oil	
15	2012	Reindeer	Norwegian Institute of Public Health
		Halibut	
		Cod liver oil	

(continued)

Table 17 (continued)

No	Year	Matrix	Organzier
16	2012	Whole egg	EU-RL for Dioxins and PCBs in Feed and Food
		Egg yolk powder	
17	2013	Poultry	Norwegian Institute of Public Health
		Crab Meat	
		Boiled Egg	
18	2013	Milk powder	EU-RL for Dioxins and PCBs in Feed and Food
		Milk fat	
19	2014	Pork	Norwegian Institute of Public Health
		Herring	
		Milk	
20	2014	Cod liver	EU-RL for Dioxins and PCBs in Feed and Food
		Fish liver oil	
21	2015	Beef	Norwegian Institute of Public Health
		Salmon	
		Cheese	
22	2015	Olive oil	EU-RL for Dioxins and PCBs in Feed and Food
		Palm oil	
23	2016	Sheep liver	Norwegian Institute of Public Health
		Salmon	
		Fish oil	
24	2016	Halibut	EU-RL for Dioxins and PCBs in Feed and Food
		Fish oil	
25	2016	Fish	UNEP
		Human milk	
26	2017	Sheep meat	Norwegian Institute of Public Health
		Cod liver	
		Herring	
27	2017	Liver of Cattle	EU-RL for Dioxins and PCBs in Feed and Food
28	2018	Reindeer	Norwegian Institute of Public Health
		Salmon	
		Fish oil	
29	2018	Beef	EU-RL for halogenated POPs in Feed and Food
30	2018	Fish Human milk	UNEP
31	2019	Veal Herring Brown meat	Norwegian Institute of Public Health
32	2019	Egg yolk powder	EU-RL for halogenated POPs in Feed and Food

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Analysis and Quality Control of WHO- and UNEP-Coordinated Human Milk Studies 2000–2019: Organochlorine Pesticides and Industrial Contaminants

Björn Hardebusch, Julia Polley, Benjamin Dambacher, Karin Kypke, and Ralf Lippold

Abstract

The analytical method for determination of nonpolar organochlorine pesticides and industrial contaminants in human milk comprises extraction of lipids, the use of three internal standards, two chromatographic separation steps for clean-up and gas chromatography on various columns of different polarity and different detectors (GC-ECD, GC-MS/MS). The more polar analytes were determined applying the QuEChERS method and HPLC-MS/MS measurement.

As accredited laboratory since 1998, a comprehensive quality control programme was applied to prove the long-term reliability of results of the WHO/UNEP-coordinated surveys on human milk performed between 2000 and 2019. The concept comprised numerous quality control samples (spiked samples for quality control charts and left-over samples from proficiency tests as in-house reference material) and participation in 53 proficiency tests covering test samples of food of animal origin, plant oils and standard solutions. Trueness calculated from the spiked quality control samples and expressed as median recovery for 29 analytes was 99% (range 86%–110%), and the precision expressed as the median of the coefficient of variation (CV) was 12% (range 0.7%–31%). Trueness calculated from in-house reference material (375 results for 41 consensus values) was on average 96% with a precision expressed as CV of 7.6%. Finally, the results of 53 proficiency tests were used to derive trueness for 23 analytes as deviation from the assigned value; this deviation was on average 14% (range 6%–27%). As conclusion of various approaches of analytical quality control, for the 2000–2019 period the long-term trueness was on average in the range 85%–95% with a precision on average better than 15%.

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_5

The general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025, the analytical criteria for analyses of organochlorine pesticides and industrial contaminants in feed and food required by EU legislation and the criteria for monitoring information for the Parties to the Stockholm Convention were met.

Keywords

Analysis · Organochlorine pesticides · Organochlorine industrial contaminants · Long-term quality control · Trueness · Precision · Human milk · Global WHO/UNEP studies

1 Introduction

In a series of articles in this compendium, an overview of the World Health Organization (WHO) and United Nations Environment Programme (UNEP)-coordinated exposure studies on persistent organic pollutants (POPs) in human milk and their link to the Stockholm Convention on POPs is given in the introduction (Part I) (Malisch et al. 2023a). Part II presents the analytical methods, Part III the results and discussion, including for organochlorine pesticides and industrial contaminants (Malisch et al. 2023b), Part IV the assessments, including time trends for selected organochlorine pesticides (Malisch et al. 2022) and Part V the summary and conclusions.

The references used for the review on findings of POPs in human milk in Part I (Fürst 2023) show a wide range of analytical methods used for the determination of organochlorine pesticides and industrial contaminants. For official food control in Germany, a multi-method for determination of lipophilic pesticide residues in food of animal origin was of particular interest, which was established in the 1980s (DFG S19; Specht et al. 1995). Over time and after successful interlaboratory studies this method was adopted as a European Norm (European Norm (EN) 1528) and included in the collection of official methods in Germany (German Standard § 64 LFBG a). The method for determination of nonpolar pesticide residues in cow's milk can be applied for human milk, as well. For more polar analytes, the QuEChERS-multimethod was established (Anastassiades et al. 2003), which was further enhanced to cover a broad range of matrices (Anastassiades 2006) and later included in the collection of official methods in Germany for determination in food of animal origin, as well (German Standard § 64 LFBG b).

In this chapter, the analytical methods for the determination of organochlorine pesticides (and their relevant metabolites) aldrin, chlordane, chlordecone, dicofol, DDT, dieldrin, endosulfan, endrin, heptachlor, hexachlorobenzene, hexachlorocyclohexanes, mirex, pentachlorophenol/pentachloroanisole and toxaphene and of the industrial chemicals hexachlorobutadiene and pentachlorobenzene are presented, including the internal and external quality control.

2 Materials and Methods

The protocols for collection of samples and a general overview on participating countries with regional distribution and temporal differentiation are given in Part I (Malisch et al. 2023a). After collection of a large number of individual samples fulfilling protocol criteria, pooled samples were prepared considered to be representative for a country or subpopulation at the time of the sampling. A total of 82 countries submitted pooled samples during one or more of the five studies conducted from 2000 to 2019. 163 pooled samples were analysed for organochlorine pesticides and industrial chemicals (Malisch et al. 2023b), applying analytical methods described in this article.

2.1 Determination of Nonpolar Substances According to EN1528 (DIN EN 1528-1:1997-1-4)

For the determination of residues of nonpolar organochlorine pesticides and contaminants, a method based on the European Norm 1528 was used, which is also part of the official collection of test methods in Germany (European Norm (EN) 1528; German Standard § 64 LFGB a). It is based on a modular structure and covers a wide range of analyte-matrix combinations (suitable for food of animal origin and human milk).

After centrifugation at 2000 g for 10 min and separation of the cream, lipids were extracted with nonpolar solvents. The supernatant cream layer was transferred into a glass beaker. By stirring, sodium sulfate was added to the cream until the mixed material was of powdery consistence. This powder was then extracted 3–4 times with *n*-hexane by thorough stirring with a glass rod and filtering the extract. The solvent was evaporated to give the extracted lipids.

As internal standards, PCB 28 (2,4,4′-trichlorobiphenyl), triphenylphosphate and PCB 209 (decachlorobiphenyl) were used. After addition of the internal standards, up to 0.5 g lipids were separated by gel chromatography using polystyrene gel (Bio-Beads S-X3; column length 740 mm, 20 mm i.d., filling level 500–500 mm; cyclohexane/ethyl acetate mixture [1:1, v/v] used as eluent). After concentration to give a volume of about 2–3 mL, the eluate was further concentrated with iso-octane added and evaporated to about 1 mL. In order to completely remove the ethyl acetate additional isooctane was added and the mixture re-concentrated.

Chromatography on a small column of partially deactivated silica gel was performed as the final clean-up step. Silica gel (70–230 mesh) was heated overnight at 130 °C and allowed to cool in a desiccator. After adding 1.5% of water, it was shaken for 30 min and then stored in a tightly sealed container. The glass chromatographic column (length 365 mm, 10 mm i.d.) was packed with 1 g of deactivated silica gel. After pre-washing with 2x5 ml hexane, the isooctane solution was loaded onto the silica column and the analytes of interest were eluted by 10 ml toluene.

The toluene fraction was evaporated to a small volume (avoiding complete dryness) and immediately taken up with cyclohexane to the intended final volume for determination by various gas chromatographic (GC) methods using capillary GC with Electron Capture Detector (ECD) and tandem mass spectrometry (MS/MS). GC-ECD measurements were performed on various instruments, including, e.g., Trace Ultra Thermo and Trace 1310 GC Thermo, with injection on two capillaries of different polarity in parallel (PS088 and DB1701). GC-MS/MS triple quadrupole mass spectrometers were used for confirmation (e.g. Chromtech Evolution GC-MS/MS, Agilent 7000 TQ GC-MS/MS) with chromatography on HP-5MS (Ultra inert 30 m, 0.25 mm i.d., 0.25 µm film thickness).

2.2 Analysis of Chlordecone and Pentachlorophenol According to the QuEChERS Method

The more polar analytes, chlordecone and pentachlorophenol *(pentachloroanisole as recommended analyte in human milk was determined by the above presented method for nonpolar substances),* were determined by the QuEChERS (**Qu**ick **E**asy **Ch**eap **E**fficient **R**obust and **S**afe) method (Anastassiades et al. 2003; Anastassiades 2006). Initially the method was developed for food of plant origin. Later the method was expanded to food matrices of animal origin like meat, egg and milk, as well (German Standard §64 LFGB b).

10 g of sample were weighed into a 40 mL centrifuge tube, fortified with internal standard (^{13}C-labelled chlordecone and ^{13}C-labelled pentachlorophenol (PCP)) and 10 mL of acetonitrile was added. By vigorous shaking, the analytes were extracted in a single-phase extraction. After addition of salts (4 g magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate) for separation of water and buffering, the mixture was shaken vigorously again and centrifuged. The acetonitrile phase was separated.

Removal of residual water and clean-up were performed simultaneously by solid phase extraction (SPE) using 25 mg PSA (*P*rimary *S*econdary *A*min) per mL acetonitrile extract and 150 mg magnesium sulfate per mL acetonitrile extract ("dispersive SPE"). After shaking and centrifugation, 1 mL of the extract was concentrated by blowing down with nitrogen to 0.25 mL final volume.

The pesticide residues contained in this solution were determined by high performance liquid chromatography coupled with tandem mass selective spectrometry detector (HPLC-MS/MS in ESI negative mode) (Agilent TQ 6490) using a C18 column (length 2.1x50 mm, 1.8 µm particle size) and a gradient of water/acetonitrile for chlordecone and of 0.005% acetic acid/acetonitrile for PCP.

3 Analytical Criteria

Regulation (EC) No 396/2005 sets Maximum Residue Levels (MRLs) for residues of pesticides in feed and food, including a number of banned active substances, e.g. certain organochlorine pesticides (Regulation (EC) No 396/2005). The methods of analysis for determination of pesticide residues shall comply with the criteria set in provisions of Community law for official controls of food and feed. Technical guidelines describe the method validation and analytical quality control (AQC) requirements to support the validity of data (DG-SANTE 2021). This document is updated on a biennial basis. The procedures and criteria of these "Pesticide Guidelines" were applied also for analysis of the human milk samples of the WHO/UNEP-coordinated exposure studies. In addition, the recommendations of the guidance on the Global Monitoring Plan for POPs on analytical methodology and quality control were met (UNEP 2019).

4 Quality Control

Accuracy is a function of systematic errors and random components. A comprehensive quality control programme was applied to prove the long-time reliability of results over many years, for most parameters between 2000 and 2019. With each series of samples, a chemical blank value and at least one quality control sample (fortified "blank sample" of an uncontaminated pork fat matrix) were included. Possible systematic errors were checked by participation in proficiency tests of any kind of matrix and by analysis of surplus material of plant and animal origin oil remaining from proficiency tests. This validation should guarantee maximum accuracy and is part of the general quality control programme applied in the daily routine for analysis of all kinds of samples, as required for an accredited laboratory for the official food control. Results of the quality control samples, proficiency tests and remainder material samples are presented in the following.

4.1 Quality Control Samples

The comprehensive quality control programme included fortified samples of an uncontaminated pork fat matrix at various concentration levels, which were monitored in quality control charts. Furthermore, remainder material from proficiency test material of various oils of plant and animal origin with consensus values for organochlorine pesticides was used for quality assurance in routine analyses. Analyses were performed by different operators using different chemicals over a long time and data collected in separate runs—therefore, these quality control data collected under intermediate conditions are much more robust than data from a single validation when one technician performs repeated analyses under the same conditions using the same chemicals in one sequence.

According to the "pesticides guidelines" (DG-SANTE 2021), acceptable limits for individual recovery results should usually be within the range of the mean recovery +/− 2x RSD. Mean recoveries from initial validation should be within the range 70–120%. A practical default range of 60–140% (control level range) may be used for individual recoveries in routine analysis. In principle, recoveries outside this default range of 60–140% would require re-analysis of the batch, but the results may be acceptable in certain justified cases. For example, if the individual recovery is unacceptably high and no residues are detected, it is not necessary to re-analyse the samples to prove the absence of residues. However, consistently high recoveries or RSDs outside ±20% must be investigated. In practise, as requested by DIN EN ISO/IEC 17025 for accredited laboratories (European Norm (EN) 17025), root cause analysis and corrective measures were initiated and further quality assurance measures were taken (e.g. repeated analyses with standard addition), when the mean recovery +/− 1x RSD was outside the range 70–120% (warning level range). The results of the quality control charts for the analytes of interest are shown in the following to illustrate the extensive compliance with the acceptable limits and exceedances in few cases.

4.1.1 Nonpolar Substances (Method EN1528; Spiked Samples)

Results of the quality control charts obtained over many years from uncontaminated spiked pork fat matrix samples show the reliability of the determination of the nonpolar organochlorine analytes determined by the DIN EN 1528-1:1997-1-4 method (European Norm (EN) 1528) for the pesticides aldrin, chlordane, DDT, dicofol, dieldrin, endosulfan, endrin, heptachlorepoxideee, hexachlorobenzene, hexachlorocyclohexanes, mirex and toxaphene, furthermore for pentachloroanisole as main metabolite of pentachlorophenol and for the industrial chemical hexachlorobutadiene.

Aldrin

Aldrin is rapidly metabolized to dieldrin and is not normally found in humans; it was not detected in any sample (<0.5 µg/kg lipid) (Malisch et al. 2023b). The determination of aldrin in a total of 22 quality control samples between 2016 and 2019 proves the good recoveries of this analyte (Fig. 1). The mean recovery from all quality control samples covering concentrations of 4, 20, 40 and 100 µg/kg lipid was 98.9% with a coefficient of variation (CV) of 8.8%. The annual mean recovery including the CV was within the warning limits in the respective years, which reflects successful quality assurance of the analytical results.

Chlordane

According to the residue definition for pesticides in food, the sum parameter "chlordane complex" comprises *cis*-chlordane (= "α-chlordane") and *trans*-chlordane (= "γ -chlordane") (both more relevant for food of plant origin) and the metabolite oxychlordane (relevant for food of animal origin; also the relevant parameter for human milk). Between 2005 and 2019, a total of 139 quality control samples were analysed for oxychlordane as the most important analyte of the

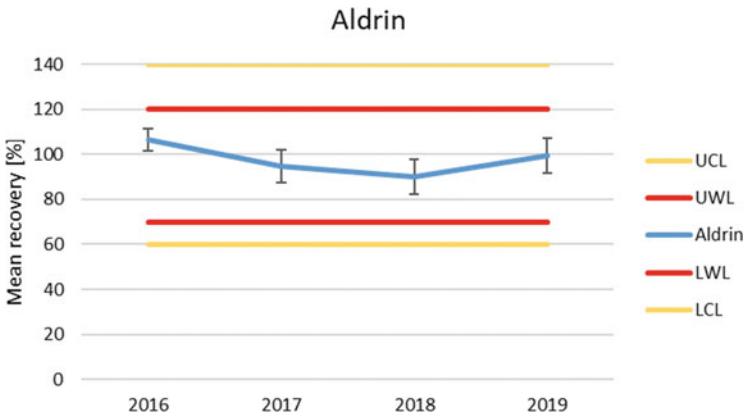

Fig. 1 Quality control chart for aldrin for the period 2016 to 2019. 22 quality control samples (uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg aldrin/kg lipid, respectively) were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

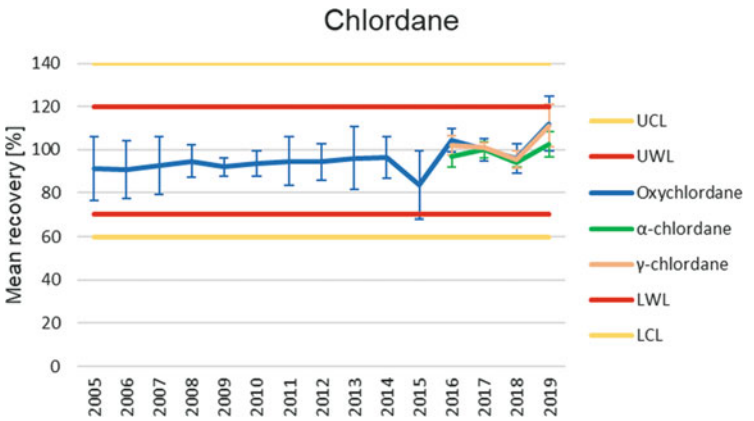

Fig. 2 Quality control chart for (i) oxychlordane based on 139 quality control samples for the period 2005 to 2019 and (ii) for α-chlordane and γ-chlordane based on 23 quality control samples for the period 2016 to 2019. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg chlordane/kg lipid, respectively, were analysed alternatingly. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

chlordane complex. Since 2016, additional 23 quality control samples were included to cover α-chlordane and γ-chlordane. The mean recovery from all quality control samples in these years was within the warning limits (Fig. 2). The mean recovery for all samples for oxychlordane was 94.5% with a CV of 12.9%. The mean recovery for

all samples for α-chlordane was 99.0% with a CV of 5.4%. The mean recovery for all samples for γ-chlordane was 103.0% with a CV of 8.2%.

DDT

Between 2000 and 2019, a total of 1192 quality control samples were analysed for p, p'-DDE as most important parameter for the sum parameter "DDT complex": In most of the human milk samples, p,p'-DDE contributed about 95%, while p,p'-DDT contributed about 5% to the sum total, and concentrations of o,p'-DDD, p,p'-DDD and o,p'-DDE were below the limit of quantification (0.5 µg/kg lipid) (Malisch et al. 2023b). In addition to p,p'-DDE, from 2015 to 2019, a total of 298 quality control samples were analysed also for o,p-DDT and p,p'-DDT, and from 2016 to 2019, a total of 31 quality control samples for p,p'-DDD. Figure 3 illustrates the mean recovery of all quality control samples in the respective year. The mean recoveries for p,p'-DDE, o,p'-DDT and p,p'-DDD were within the warning limits, but p, p'-DDT exceeded the UWL on few occasions.

In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when recoveries were outside the warning levels and further quality assurance measures were taken (e.g. repeated analyses with standard addition), to ensure that the mean recovery for the analytes was again successfully within the warning limits. The mean recovery for all samples for p,p'-DDE in the period 2000–2019 was 96.2% with a CV of 14.3%. The mean recovery for all samples for the sum parameter "DDT complex" in the period 2016–2019 was 108.1% with a CV of 15.3%.

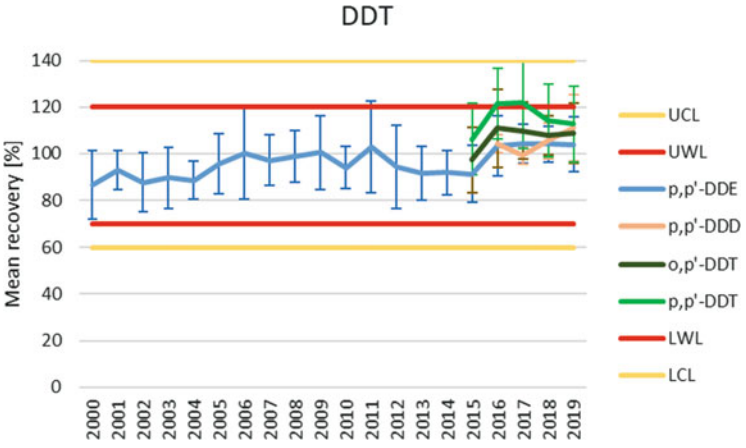

Fig. 3 Quality control chart for DDT used from 2000 to 2019 (1192 quality control samples for p, p'-DDE, 298 quality control samples for o,p'-DDT and p,p'-DDT and 31 quality control samples for p,p'-DDD). Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg DDT/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Dicofol (4,4′)

Dicofol was listed in the Stockholm Convention in 2019 and, in agreement with UNEP the analytical spectrum for the 2016–2019 survey included this compound in order to have a complete picture of all 30 POPs as covered by the Convention until 2019 (Malisch et al. 2023b). Between 2018 and 2019, a total of 13 quality control samples were analysed for the dicofol (4,4′) (Fig. 4). The mean recovery from all samples was 98.4% with a CV of 5.9% and within the warning limits.

Dieldrin

The determination of dieldrin in a total of 256 quality control samples between 2015 and 2019 proves the good recoveries of this analyte (Fig. 5). The mean recovery derived from quality control samples was 103.0% with a CV of 11.5% and was always within the warning limits in the respective years.

Endosulfan

Technical-grade endosulfan, which contains a number of related isomers, was listed in the Stockholm Convention in 2011. The sum parameter endosulfan complex comprises the analytes α-endosulfan, β-endosulfan and endosulfan-sulfate. Between 2005 and 2019, a total of 139 quality control samples were analysed for endosulfan-sulfate. From 2016 to 2019, a total of 24 quality control samples were analysed for β-endosulfan, and from 2017 till 2019, an additional 19 quality control samples for

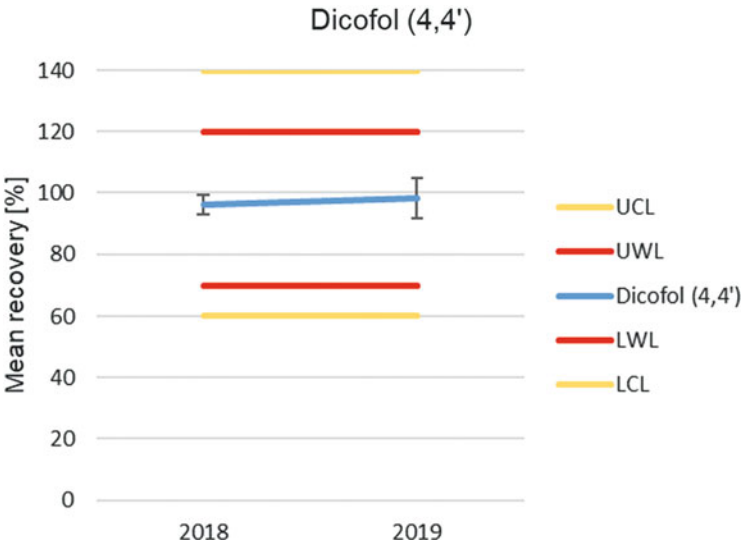

Fig. 4 Quality control chart for dicofol (4,4′) used from 2018 to 2019. 13 quality control samples (uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg dicofol (4,4′)/kg lipid, respectively) were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

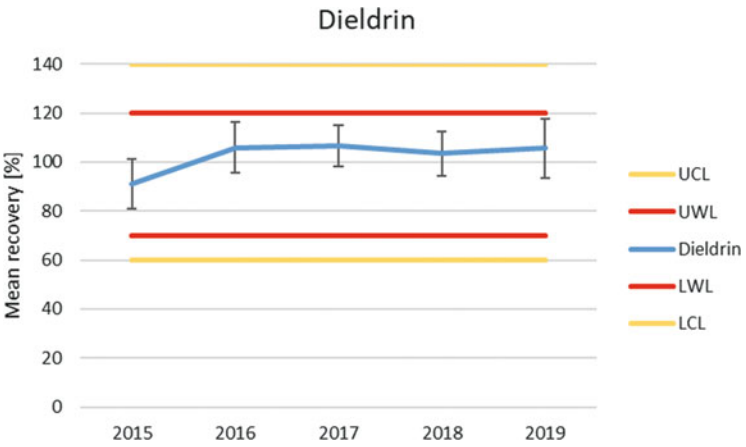

Fig. 5 Quality control chart for dieldrin for the period 2015 to 2019. 256 quality control samples (uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg dieldrin/kg lipid, respectively) were analysed alternately over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

α-endosulfan were analysed. Figure 6 illustrates the mean recovery from the quality control samples in the respective year, which were within the warning limits. The mean recovery for all samples for endosulfan-sulfate for the period 2005–2019 was 102.2% with a CV of 15.6%. The mean recovery for all samples for the sum parameter endosulfan complex for the period 2017–2019 was 95.4% with a CV of 19.5%.

Endrin

Between 2016 and 2019, a total of 22 quality control samples were analysed for endrin. Figure 7 illustrates the mean recovery from all quality control samples in the respective years. The mean recovery from all quality control samples was 100.5% with a CV of 13.4% and therefore within the warning limits.

Heptachlorepoxide

Heptachlor is rapidly metabolized to heptachlorepoxideee. In humans only cis-heptachlorepoxideee is normally found. Between 2003 and 2019, a total of 365 quality control samples were analysed for cis-heptachlorepoxideee. Between 2017 and 2019, 19 quality control samples were analysed for trans-heptachlorepoxideee, as well. Figure 8 illustrates the mean recovery from all quality control samples in the respective years. The mean recoveries for cis- and trans-heptachlorepoxideee were nearly identical and within the warning limits. The mean recovery for all samples for cis-heptachlorepoxideee in the period 2003–2019 was 98.9% with a CV of 13.1%. The mean recovery for all samples for trans-heptachlorepoxideee in the period 2017–2019 was 101.7% with a CV of 10.0%.

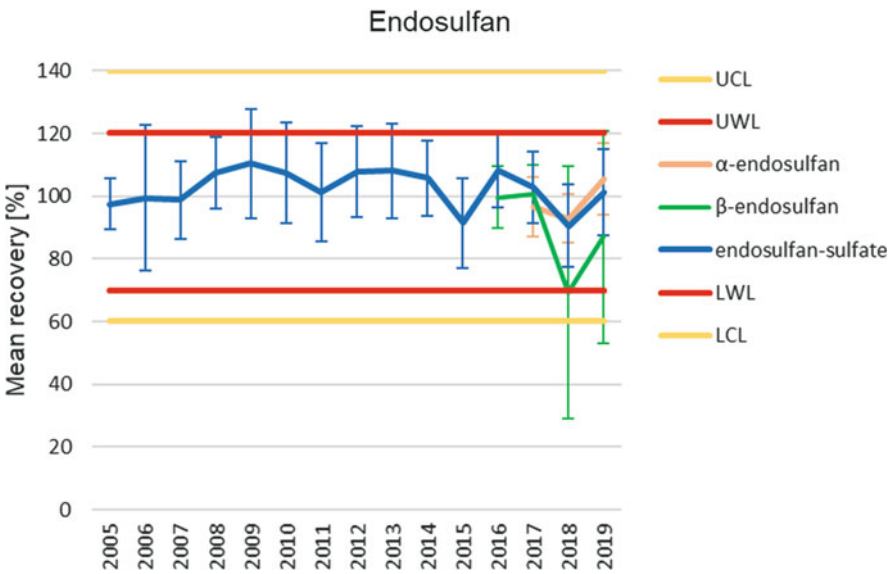

Fig. 6 Quality control chart for (i) endosulfan-sulfate for the period 2005 to 2019 with 139 quality control samples, (ii) for β-endosulfan for the period 2016 to 2019 with 24 quality control samples and (iii) for α-endosulfan for the period 2017 to 2019 with 19 quality control samples. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg endosulfan/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Hexachlorobenzene

Between 2000 and 2019, a total of 1116 quality control samples were analysed for hexachlorobenzene (HCB). Figure 9 illustrates the mean recovery from all quality control samples in the respective year, which were all in the warning limits. The mean recovery for all samples for HCB in the period 2000–2019 was 85.0%, with a CV of 18.0%.

Hexachlorobutadiene

Production and use of hexachlorobutadiene (HCBD) were prohibited by listing HCBD in 2015 in Annex A (Elimination) of the Stockholm Convention in 2015. HCBD can also be formed as unintentional by-product and was therefore listed in 2017 also in Annex C (Unintentional production). Therefore, analyses were performed for samples of the 2016–2019 period after validation of the analytical method. In 2018 and 2019, a total of 12 quality control samples were analysed for HCBD. Figure 10 illustrates the mean recovery from all quality control samples in the respective year. The mean recovery was 107.8% with a CV of 7.6% and within the warning limits.

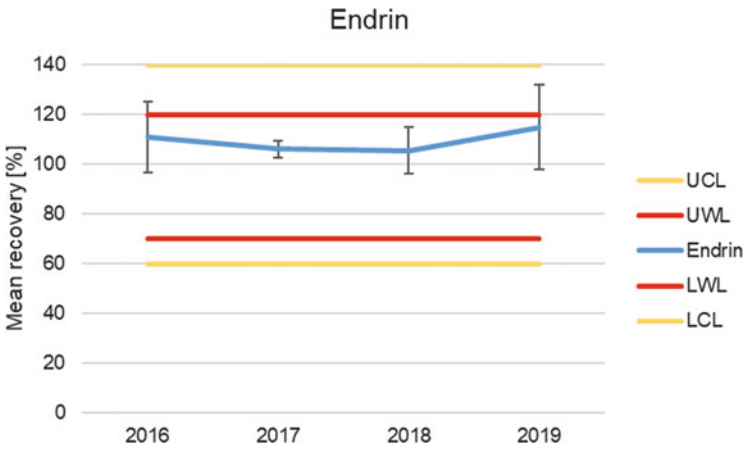

Fig. 7 Quality control chart for endrin for the period 2016 to 2019 (22 quality control samples). Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg endrin/kg lipid, respectively, were alternatingly analysed over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

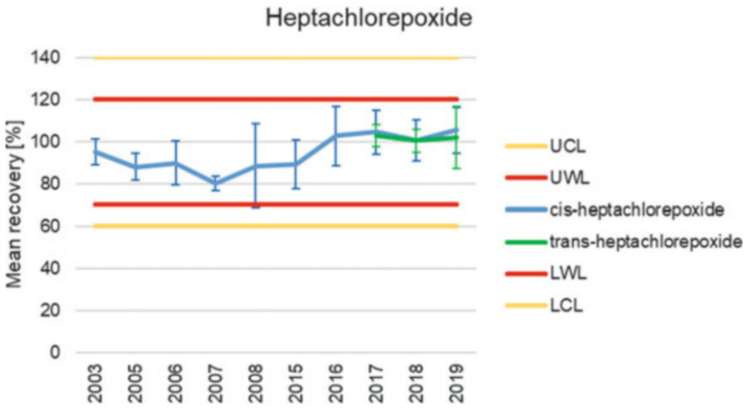

Fig. 8 Quality control chart for cis-heptachlorepoxidee for the period 2003 to 2019 (365 quality control samples) and for trans-heptachlorepoxidee for the period 2017 to 2019 (19 quality control samples). Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg cis-heptachlorepoxidee/kg lipid and in the period 2017–2019 in addition spiked with 2, 10, 25 and 50 µg trans-heptachlorepoxidee/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

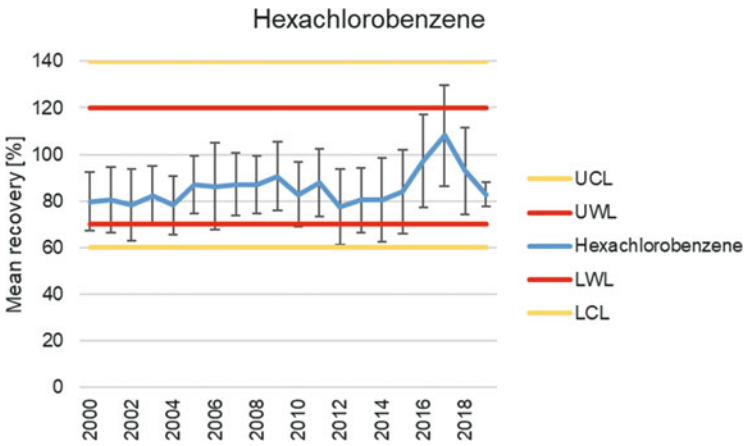

Fig. 9 Quality control chart for hexachlorobenzene for the period 2000 to 2019. 1116 lipid quality control samples were analysed during this period. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 μg HCB/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

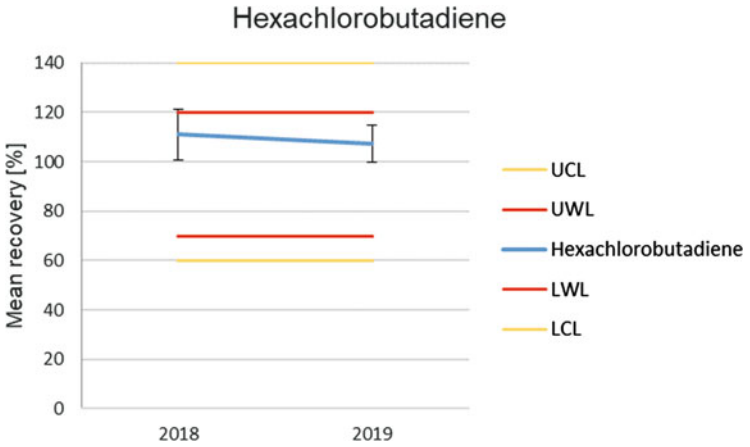

Fig. 10 Quality control chart for hexachlorobutadiene for the period 2018 to 2019. 12 lipid quality control samples were analysed during this period. Uncontaminated pork fat matrix samples spiked with 4 μg HCBD/kg lipid, respectively, were analysed over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH)

Technical-grade hexachlorocyclohexane (HCH) is a mixture mainly of three isomers comprising about 65–70% α-HCH, 7–20% β-HCH and 14–15% γ-HCH. These

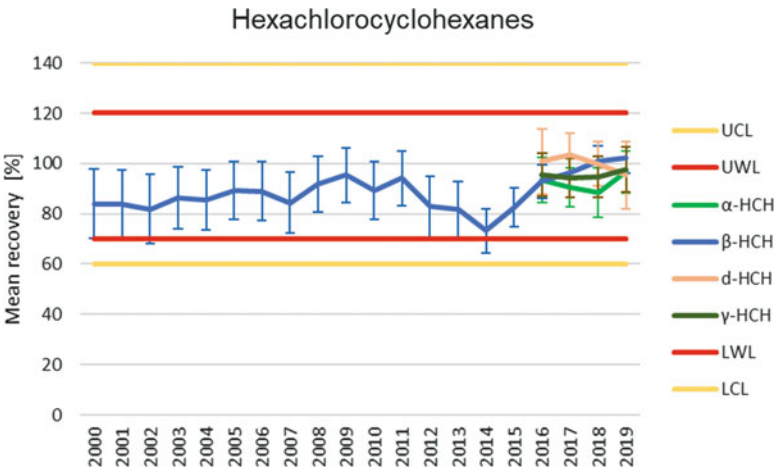

Fig. 11 Quality control chart for hexachlorocyclohexanes. In the period 2000 to 2019, 1163 quality control samples were analysed for β-HCH, in the period 2016 to 2019, 185 samples for α–HCH, 218 samples for δ-HCH and 55 samples for γ-HCH. Uncontaminated pork fat matrix samples spiked with 4, 20, 40 and 100 µg HCH/kg lipid, respectively, were analysed alternatingly over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

isomers were listed in 2009 in Annex A (for elimination) by the Stockholm Convention. However, the minor component δ-HCH; 6–10% was not listed. Metabolism results in the accumulation of mainly β-HCH in humans, whereas the concentrations of α-HCH and γ-HCH were below the limit of quantification (< 0.5 µg/kg lipid) in most human milk samples (Malisch et al. 2023b). Therefore, the main focus of quality control in the period 2000–2019 was on β-HCH; α-, γ- and δ-HCH were added for particular control during the 2016–2019 study.

Between 2000 and 2019, a total of 1163 quality control samples were analysed for β-HCH. In the period 2016–2019, additional 185 quality control samples were included for the determination of α-HCH, 55 samples for γ-HCH and 218 samples for δ-HCH. Figure 11 illustrates the mean recovery from all quality control samples in the respective year. The mean recoveries from all quality control samples were within the warning limits. The mean recovery for all samples for β-HCH in the period 2000–2019 was 89.0% with a CV of 16.5%. The mean recovery for all samples for α-, β-, γ-, δ-HCH in the period 2016–2019 was 96.4% with a CV of 11.3%.

Mirex

Between 2000 and 2019, a total of 1437 quality control samples were analysed for mirex. Figure 12 illustrates the mean recovery in the respective year, which were within the warning limits. The mean recovery for all samples for mirex in the period 2000–2019 was 93.3% with a CV of 13.9%.

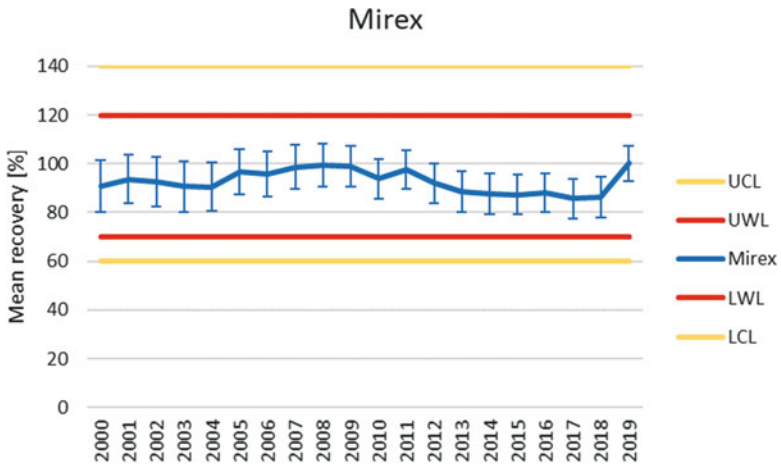

Fig. 12 Quality control chart for mirex for the period 2000 to 2019. 1437 lipid quality control samples were analysed for mirex as representative analyte and internal standard. Uncontaminated pork fat matrix samples spiked with concentration level of 4 µg mirex/kg lipid and 40 µg mirex as internal standard/kg lipid were determined over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

Pentachloroanisole

Between 2018 and 2019, a total of 11 quality control samples were analysed for pentachloroanisole. Figure 13 illustrates the mean recovery from all quality control samples in the respective year. The mean recovery from all quality control samples was 87.9% with a CV of 5.9% and in these years within the warning limits.

Toxaphene

Between 2000 and 2019, a total of 160 quality control samples were analysed for the toxaphene marker compounds Parlar 26, Parlar 50 and Parlar 62. Figure 14 illustrates the mean recovery from all quality control samples in the respective year. Except some outliers in the period 2001–2002 for Parlar 62, the mean recoveries from these quality control samples were within the warning limits. In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when recoveries were outside the warning levels and further quality assurance action was taken (e.g. repeated analyses with standard addition), to ensure that the mean recovery for all toxaphene analytes was within the warning limits. The mean recovery for the sum parameter of Parlar 26, Parlar 50 and Parlar 62 was 97.2% with a CV of 23.7%.

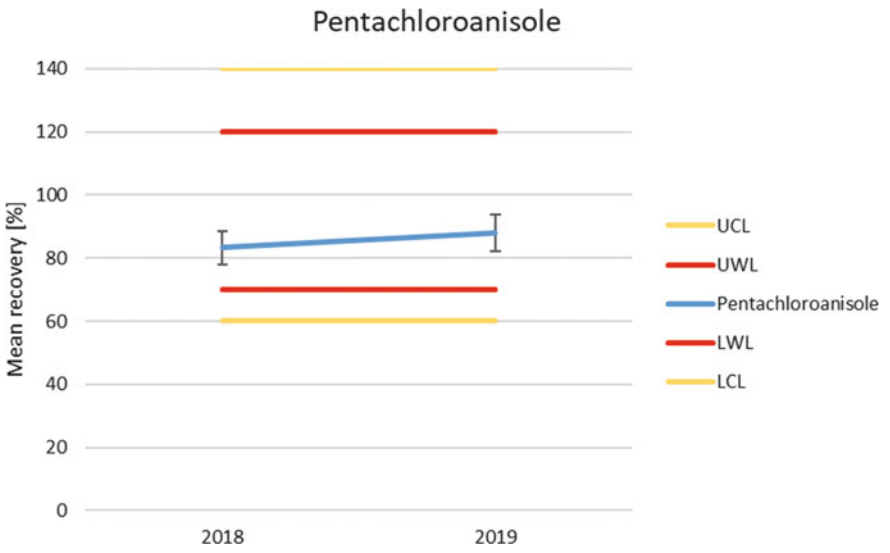

Fig. 13 Quality control chart for pentachloroanisole for the period 2018 to 2019. 11 uncontaminated pork fat matrix quality control samples spiked at 20 µg pentachloroanisole/kg lipid were analysed. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

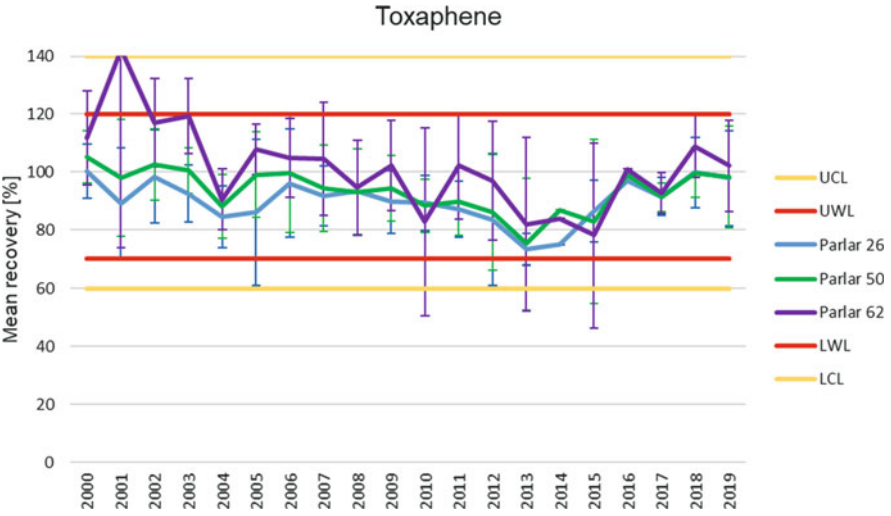

Fig. 14 Quality control chart for toxaphene for the period 2000 to 2019. 160 uncontaminated pork fat matrix quality control samples spiked at 10 µg toxaphene/kg lipid with each of the three marker compounds Parlar 26, Parlar 50 and Parlar 62 were analysed over this period. The recovery rate (%) shown on annual basis was calculated as mean of the respective samples; the corresponding coefficient of variation (%) is illustrated by error bars. *UWL* upper warning level, *LWL* lower warning level, *UCL* upper control level, *LCL* lower control level

4.1.2 Chlordecone and Pentachlorophenol (QuEChERS Method; Spiked Samples)

The validation of the more polar organochlorine pesticides chlordecone and pentachlorophenol in samples of the 2016–2019 period is based on a LC-MS/MS method.

Chlordecone

For validation of chlordecone, raw cow's milk with a fat content of 4% was spiked at 5 concentrations and the recovery determined by 6 replicates. The mean recovery (%) and the CV % fulfilled the requirements for analytical quality control for pesticide residue analysis (mean recovery 70–120%, CV < 20%) (DG SANTE, 2021) (Table 1).

Two human milk matrix quality control samples were fortified with 10 µg/kg fresh weight and analysed for chlordecone. The mean recovery was 92.0%.

Pentachlorophenol (PCP)

In a minimal validation of pentachlorophenol by LC-MS/MS determination, a pork muscle tissue matrix was used. The pork matrix was spiked at 2 levels and determined in 6 replicates, respectively. The requirements for analytical quality control for pesticide residue analysis (mean recovery 70–120%, CV < 20%) (DG SANTE 2021) were met (Table 2).

Two human milk matrix quality control samples were spiked at 10 µg/kg fresh weight and analysed for pentachlorophenol. The mean recovery was 96.6%.

4.1.3 Summary: Trueness and Precision Derived from Spiked Quality Control Samples

Table 3 summarizes the results of the quality control data for spiked samples with regard to trueness (expressed as mean recoveries in %) and precision (expressed as CV in %). The trueness for all analytes was on average 99% (range 86%–110%) and the precision on average 11.5% (range 0.7%–31.4%). In conclusion, based on quality control samples, the applied analytical methods achieved a very good long-term performance for trueness and precision over the 2000–2019 period.

Table 1 Validation data for chlordecone in raw cow's milk

µg/kg milk	µg/kg milk fat	Recovery [%]	(CV) [%]
0.01	0.25	98.5	11.8
0.02	0.5	100.1	12.8
0.05	1.25	101.9	8.1
0.1	2.5	99.3	6.0
0.2	5.0	103.1	4.8

Table 2 Validation data of pentachlorophenol (PCP) in pork muscle matrix milk

µg PCP/kg pork muscle	Average [µg PCP /kg pork muscle]	Recovery [%]	CV [%]
10	9.7	96.7	2.8
50	51.3	102.6	1.6

Table 3 Mean recoveries (%) and CVs (%) for 16 parameters with 29 analytes

Analyte	Mean recoveries (%)	CV (%)
Aldrin	98.9	8.8
Chlordecone	92.9	3.4
Chlordane		
Oxychlordane	94.5	12.9
α-chlordane	99	5.4
γ-chlordane	103.8	8.2
DDT		
p,p'-DDE	96.2	14.3
p,p'-DDT	107.3	13.8
o,p'-DDT	107.3	13.8
p,p'-DDD	105.7	9.4
Dicofol	98.4	5.9
Dieldrin	103	11.5
Endosulfan		
Endosulfan-sulfate	102.2	15.6
α-endosulfan	99.8	11.3
β-endosulfan	90	26.4
Endrin	110.5	13.4
Heptachlorepoxide		
cis-heptachlorepoxidee	98.9	13.1
trans-heptachlorepoxidee	101.7	10
Hexachlorobenzene	85.7	18
Hexachlorobutadiene	107.8	7.6
Hexachlorocyclohexanes		
α–HCH	91.1	9.2
β-HCH	89	16.5
γ-HCH	95.6	8.5
δ-HCH	99.6	11.7
Mirex	100.7	6.9
Pentachloroanisole	87.9	5.9
Pentachlorophenol	96.6	0.7
Toxaphene		
Parlar 26	91.6	15.4
Parlar 50	95	15.8
Parlar 62	104.9	31.4
Min	85.7	0.7
Median	98.9	11.5
95% Percentile	107.6	23.0
Max	110.5	31.4
N_1 = number of analytes	29	
N_2 = number of samples	6465	

4.2 Remainders of Samples from Proficiency Tests as in-house Reference Material

10 samples (plant oil and animal origin oil spiked with several organochlorine pesticides) from excess materials provided for proficiency tests with defined consensus values were used as in-house quality control samples. Trueness calculated from 375 results for 41 consensus values was on average 96% and the precision expressed as CV 7.6% (Table 4).

4.3 Participation in Proficiency Tests for Pesticide Residues

Between 2000 and 2019, CVUA Freiburg participated in 53 proficiency tests (PTs) for determination of pesticide residues in test samples mainly of food of animal origin, of plant oils and in two cases of human milk (Table 5). The analytical methods applied for these different matrices use the same modular elements. In certain PTs, standard solutions also had to be analysed. Due to the limited availability of toxaphene proficiency tests, the results of the 1999 proficiency test on toxaphenes in a fish (halibut) matrix were also included as additional information.

In these proficiency tests, around 150 different analytes were determined resulting in a total of around 800 data. 26 of these analytes were organochlorine pesticides providing a total of around 170 results.

In order to summarize the evaluation of all 53 samples of these proficiency tests performed between 1999 and 2019, the results were calculated on a lipid basis, fresh weight basis or solution basis (according to the "pesticides guidelines" (DG-SANTE 2021)), regardless of the requirements for reporting data for individual proficiency tests. The general idea of this summarizing evaluation was to check how the results reported by CVUA Freiburg deviated from the respective consensus value.

Figures 15, 17, 19, 20, 22, 24, 26, 28, 30, 31 show the deviation (%) from the consensus values for all results obtained in the 53 PTs for the analytes chlordane (cis-chlordane; trans-chlordane; oxychlordane), DDT (o,p'-DDD; p,p'-DDD, o,p'-DDE; p,p'-DDE, o,p'-DDT, p,p'-DDT), dieldrin, endosulfan (α-endosulfan; β-endosulfan; endosulfan-sulfate), endrin, hexachlorobenzene, hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), heptachlorepoxide (cis-heptachlorepoxide; trans-heptachlorepoxide), mirex and toxaphene (Parlar 26, Parlar 50 and Parlar 62). In all figures, the deviations for results calculated on a lipid, fresh weight and solution basis are marked as dots, triangles and squares, respectively, as described in the table caption.

Figures 16, 18, 21, 23, 25, 27, 29, 32 illustrate the z-score values obtained in 51 of the 53 PTs. The evaluation of these 51 PTs is based on the same principle and therefore gives comparable results: The consensus values were calculated as robust mean or median from all results without obvious outliers and the z-score calculations were performed by applying a robust standard deviation or the standard deviation according to Horwitz. The results of the two PTs following the Cofino-model were not included in the comparison. In accordance with the ISO 17043, z-scores of

Table 4 Mean recoveries (%) and CVs (%) for 10 left-over oil samples of plant and animal origin

Matrix	Analyte	Consensus µg/kg	Number of values	Mean µg/kg	Recovery (%)	CV (%)
Oil	Chlordane-oxy	20.0	8	17.4	87.2	**3.0**
Oil	Chlordane-oxy	31.2	6	29.8	95.4	**7.6**
Oil	Chlordane-oxy	55.6	17	53.1	95.5	**6.8**
Oil	Chlordane-trans	43.9	6	39.8	90.6	**7.8**
Oil	Chlordane-trans	84.8	6	73.4	86.5	**1.3**
Oil	DDE-pp'	21.6	9	21.0	97.2	**11.8**
Oil	DDE-pp'	24.3	8	23.3	95.8	**7.9**
Oil	DDE-pp'	76.8	6	78.1	101.7	**5.0**
Oil	DDE-pp'	93.7	17	89.2	95.2	**6.8**
Oil	DDE-pp'	524.1	6	568.5	108.5	**18.4**
Oil	DDT-pp'	33.0	8	33.2	100.7	**3.9**
Oil	DDT-pp'	90.6	6	91.7	101.2	**10.9**
Oil	DDT-pp'	93.4	17	90.5	96.9	**8.7**
Oil	Dieldrin	10.7	6	9.57	89.4	**14.8**
Oil	Dieldrin	13.3	8	11.9	89.3	**5.1**
Oil	Dieldrin	56.5	17	53.4	94.5	**6.8**
Oil	Dieldrin	173.3	9	182.8	105.5	**6.3**
Oil	Endosulfane-α	3.80	6	3.18	83.8	**13.4**
Oil	Endosulfane-α	71.4	6	70.0	98.0	**3.4**
Oil	Endosulfane-β	6.70	6	6.00	89.6	**30.3**
Oil	Endosulfane-β	37.8	6	36.2	95.8	**5.4**
Oil	Endosulfane sulfate	26.6	6	23.5	88.3	**14.0**
Oil	Endrin	19.5	6	15.2	77.8	**5.4**
Oil	Endrin	21.5	8	15.6	72.4	**5.4**
Oil	Endrin	39.2	17	33.2	84.7	**14.9**
Oil	Endrin	44.8	6	43.9	97.9	**4.2**
Oil	Endrin	46.3	9	46.0	99.4	**11.4**
Oil	HCB	21.9	8	22.6	103.0	**7.3**
Oil	HCB	43.0	9	46.3	107.6	**12.5**
Oil	HCB	65.0	6	71.1	109.4	**7.3**
Oil	HCB	76.6	17	76.4	99.8	**8.7**
Oil	HCB	362.2	6	355.1	98.0	**16.9**
Oil	HCH-β	12.4	8	13.4	108.0	**8.5**
Oil	HCH-β	20.7	6	18.9	91.1	**8.5**
Oil	HCH-β	48.5	17	44.1	91.0	**13.6**
Oil	HCH-γ	33.7	8	32.4	96.2	**6.5**
Oil	HCH-γ	63.4	6	68.7	108.3	**3.0**
Oil	HCH-γ	67.9	6	86.0	126.6	**13.2**
Oil	HCH-γ	82.1	17	77.8	94.7	**5.7**
Oil	Heptachlorepoxide-cis	22.8	8	18.0	78.8	**5.4**
Oil	Heptachlorepoxide-cis	44.7	17	35.9	80.2	**10.1**

(continued)

Table 4 (continued)

Matrix	Analyte	Consensus µg/kg	Number of values	Mean µg/kg	Recovery (%)	CV (%)
	Min	3.8	6.0	3.2	**72.4**	**1.3**
	Median	43.9	8.0	39.8	**95.8**	**7.6**
	95% percentile	173.3	17.0	182.8	**108.5**	**16.9**
	97.5% percentile	362.2	17.0	355.1	**109.4**	**18.4**
	Max	524.1	17.0	568.5	**126.6**	**30.3**
	N	**41**	**375**			

Table 5 Overview of proficiency tests between 1999 and 2019

No	Year	Organization	Matrix
1	2000	FAPAS	Plant oil
2	2000	LVU Herbolzheim	Plant oil
3	2001	FAPAS/BgVV	Meat (poultry)
4	2002	LVU Herbolzheim	Plant oil
5	2003	FAPAS	Fish
6	2003	FAPAS	Plant oil
7	2003	FAPAS	Meat (poultry)
8	2003	LVU Herbolzheim	Solution
9	2004	FAPAS	Milk powder
10	2004	LVU Herbolzheim	Plant oil
11	2004	BVL	Meat (boiled sausage)
12	2004	FAPAS	Plant oil
13	2004	FAPAS	Milk powder
14	2004	FAPAS	Meat (poultry)
15	2005	FAPAS	Milk powder
16	2006	FAPAS	Fish meal
17	2006	LVU Herbolzheim	Plant oil
18	2007	BVL	Egg
19	2007	FAPAS	Fish
20	2008	FAPAS	Milk powder
21	2009	FAPAS	Fish
22	2009	FAPAS	Milk powder
23	2010	FAPAS	Meat (poultry)
24	2010	FAPAS	Milk powder
25	2011	FAPAS	Minced Oily Fish
26	2011	FAPAS	Plant oil
27	2012	FAPAS	Milchpulver
28	2013	FAPAS	Minced Oily Fish
29	2013	FAPAS	Pork, muscle
30	2013	BIPEA	Honey
31	2014	FAPAS	Milk powder

(continued)

Table 5 (continued)

No	Year	Organization	Matrix
32	2014	FAPAS	Animal Fat
33	2014	FAPAS	Rapeseed oil
34	2015	FAPAS	Animal fat (Pork)
35	2015	FAPAS	Fish oil
36	2015	FAPAS	Milk powder
37	2016	FAPAS	Animal fat (pork)
38	2016	FAPAS	Milk powder
39	2016	FAPAS	Infant formula
40	2016–2017	UNEP	Fish
41	2016–2017	UNEP	Human milk
42	2017	BIPEA	Egg powder
43	2017	FAPAS	Chicken egg
44	2017	FAPAS	Oily fish
45	2017	FAPAS	Olive Oil
46	2017	FAPAS	Infant formula
47	2017	FAPAS	Offal (Liver)
48	2018	ANSES	Fish
49	2018–2019	UNEP	Fish
50	2018–2019	UNEP	Human milk
51	2018	FAPAS	Infant Formula
52	2019	FAPAS	Infant Formula
53*	1999	BgVV	Halibut

* Proficiency test results for additional information for the quality measures for toxaphene

$|z| \leq 2.0$ indicate a "satisfactory" performance and generate no signal, z-scores between the range $2.0 < |z| < 3.0$ indicate "questionable" performance and generate warning signals and z-scores above $|z| \geq 3.0$ indicate "unsatisfactory" performance and generate action signals. In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when z-scores were above $|z| \geq 2.0$.

4.3.1 Chlordane

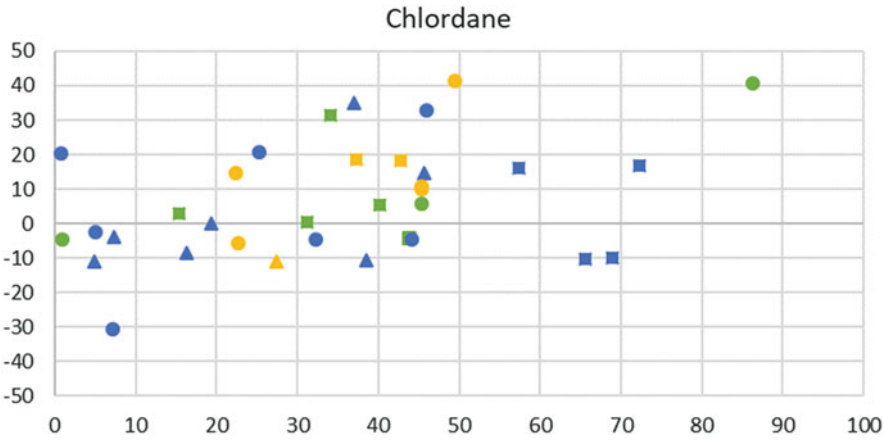

Fig. 15 Deviation (%) from the assigned values of 34 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for chlordane in proficiency tests performed between 2000 and 2019 (cis-chlordane visualized as dots, trans-chlordane as triangles and oxy-chlordane as squares)

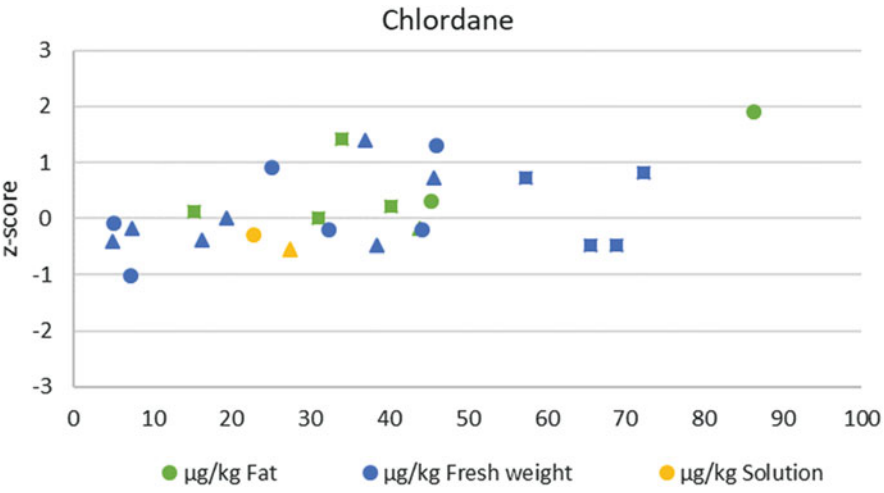

Fig. 16 Z-score of 26 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for cis-chlordane, trans-chlordane, oxy-chlordane for deviation from the assigned values of proficiency tests performed between 2000 and 2019 (cis-chlordane visualized as dots, trans-chlordane as triangles and oxy-chlordane as squares)

4.3.2 DDT

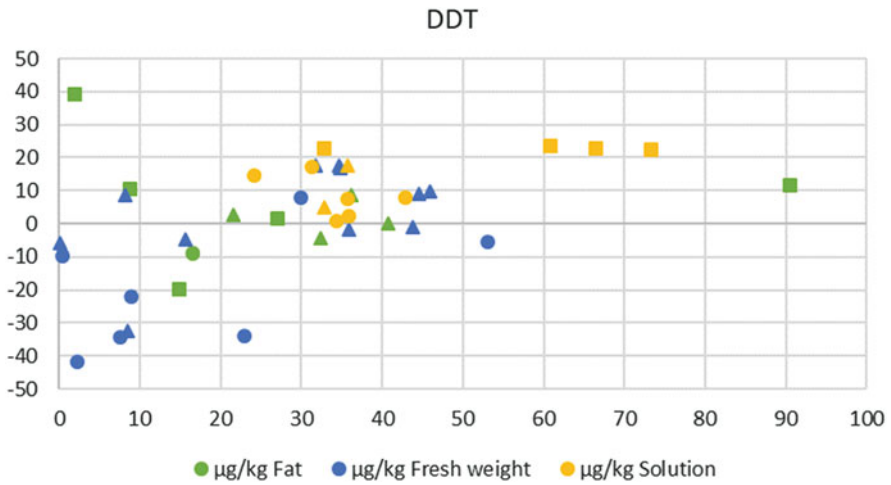

Fig. 17 Deviation (%) from the assigned values of 40 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for DDT in proficiency tests performed between 2000 and 2019 (o,p'-DDD and p,p'-DDD visualized as dots, o,p'-DDE and p,p'-DDE as triangles, o,p'-DDT and p,p'-DDT as squares)

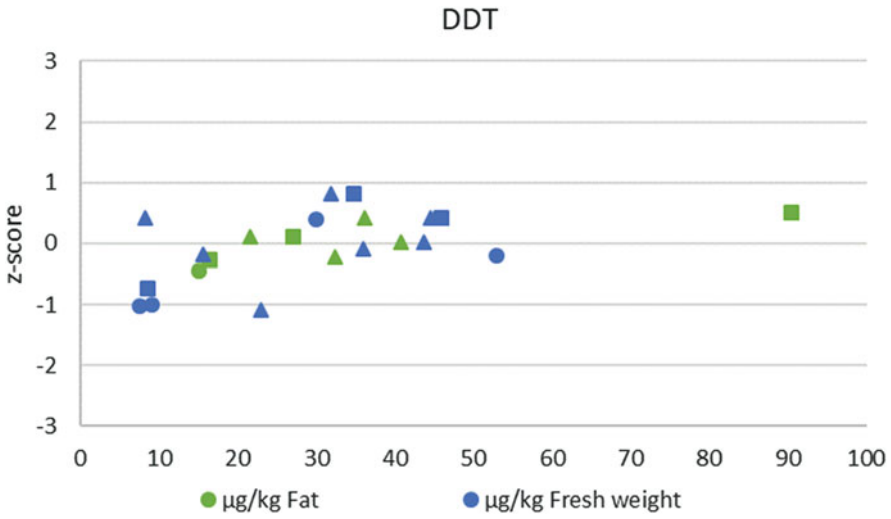

Fig. 18 Z-score of 23 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for o,p'-DDD, p,p'-DDE, o,p'-DDT and p,p'-DDT for deviation from the assigned values of proficiency tests performed between 2000 and 2019 (o,p'-DDD and p,p'-DDD visualized as dots, o,p'-DDE and p,p'-DDE as triangles, o,p'-DDT and p,p'-DDT as squares)

4.3.3 Dieldrin

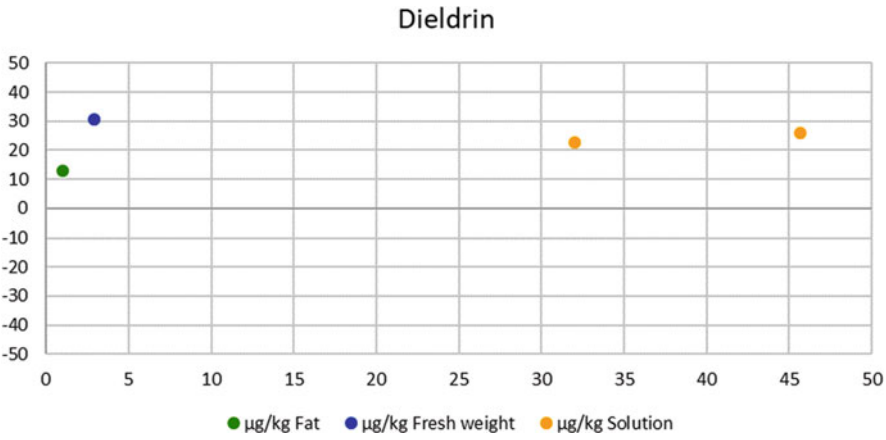

Fig. 19 Deviation (%) from the assigned values of 4 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for dieldrin in proficiency tests performed between 2016 and 2019 (no calculation of z-scores in these PTs)

4.3.4 Endosulfan

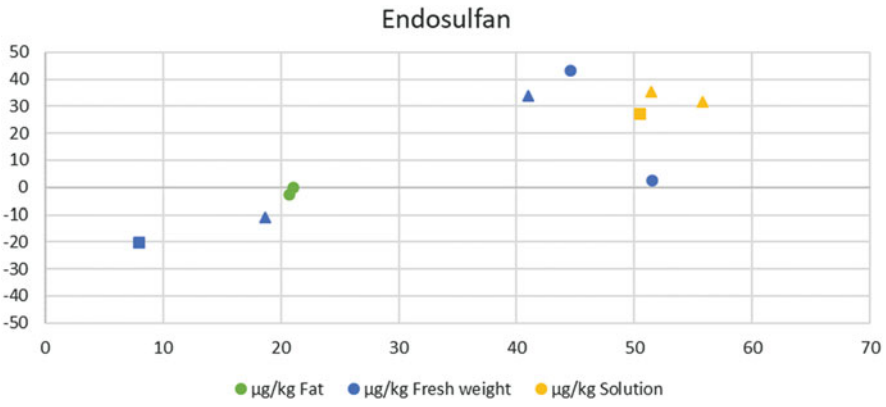

Fig. 20 Deviation (%) from the assigned values of 13 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for endosulfan in proficiency tests performed between 2000 and 2019 (endosulfan-sulfate visualized as dots, β-endosulfan as triangles, α-endosulfan as squares)

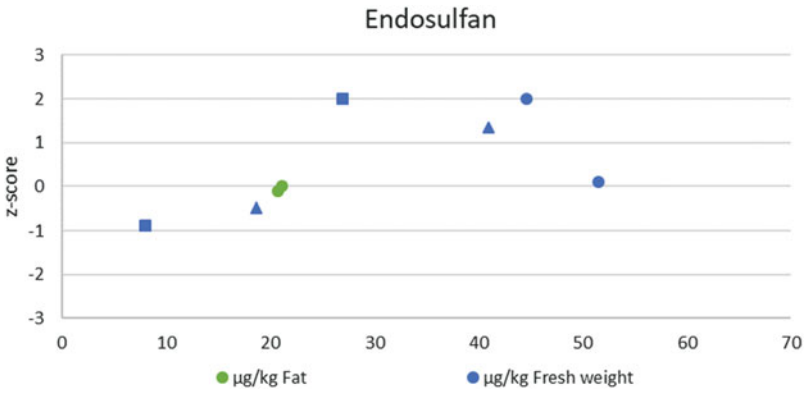

Fig. 21 Z-score of 8 results (µg/kg fresh weight and µg/kg lipid) for α-endosulfan, β-endosulfan and endosulfan-sulfate for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (endosulfan-sulfate visualized as dots, β-endosulfan as triangles, α-endosulfan as squares)

4.3.5 Endrin

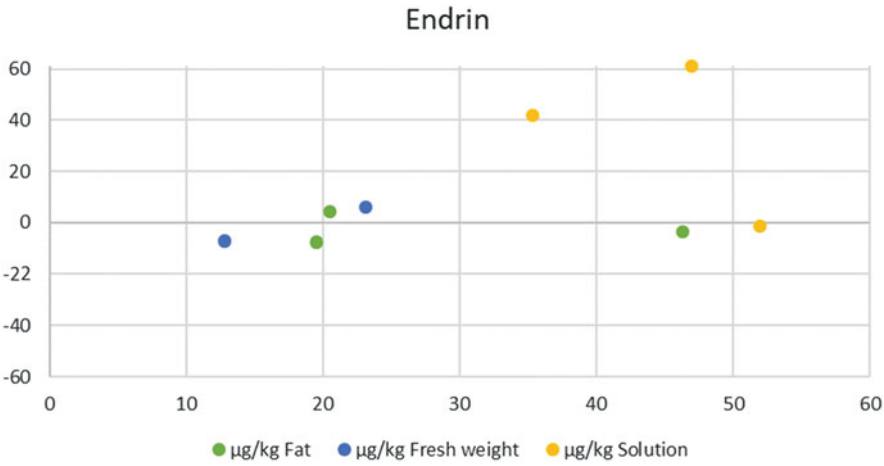

Fig. 22 Deviation (%) from the assigned values of 8 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for endrin in proficiency tests performed between 2000 and 2019

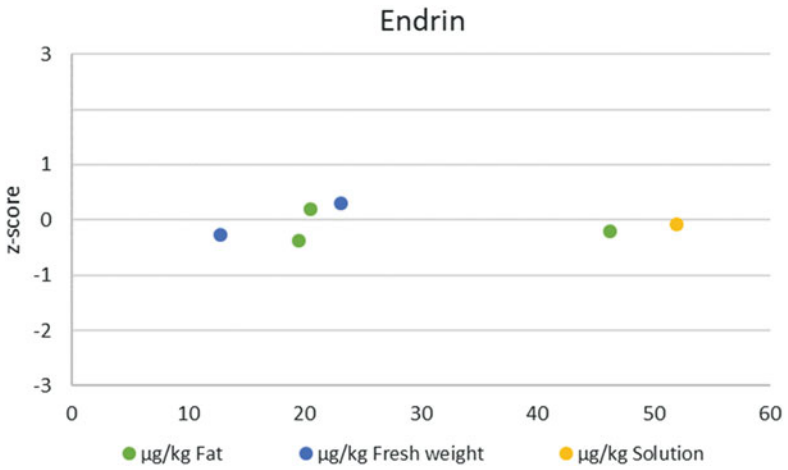

Fig. 23 Z-score of 6 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for endrin for deviation from the assigned values of proficiency tests performed between 2000 and 2015

4.3.6 Hexachlorobenzene (HCB)

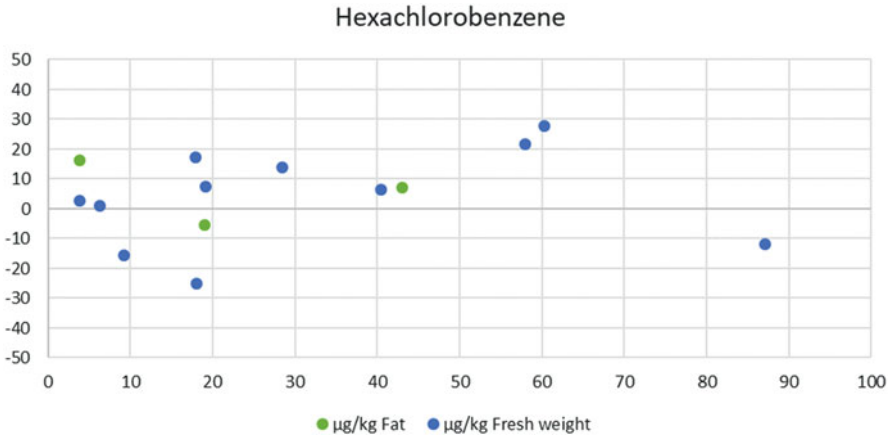

Fig. 24 Deviation (%) from the assigned values of 15 results (µg/kg fresh weight or µg/kg lipid) for hexachlorobenzene in proficiency tests performed between 2000 and 2019

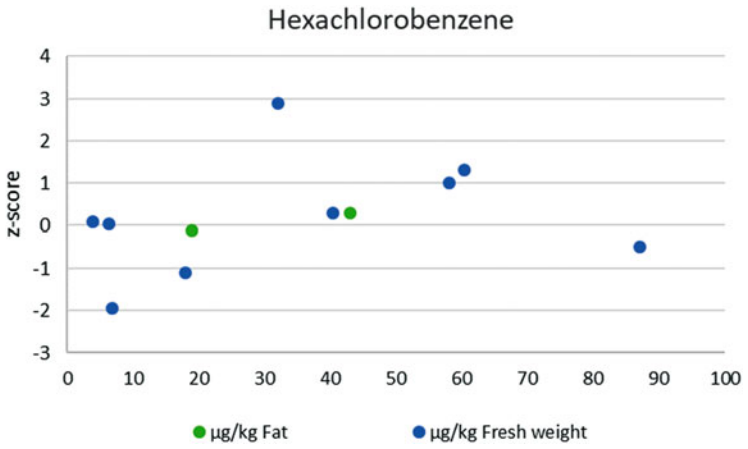

Fig. 25 Z-score of 11 results (µg/kg fresh weight or µg/kg lipid) for hexachlorobenzene for deviation from the assigned values of proficiency tests performed between 2000 and 2017

4.3.7 Hexachlorocyclohexanes (HCH)

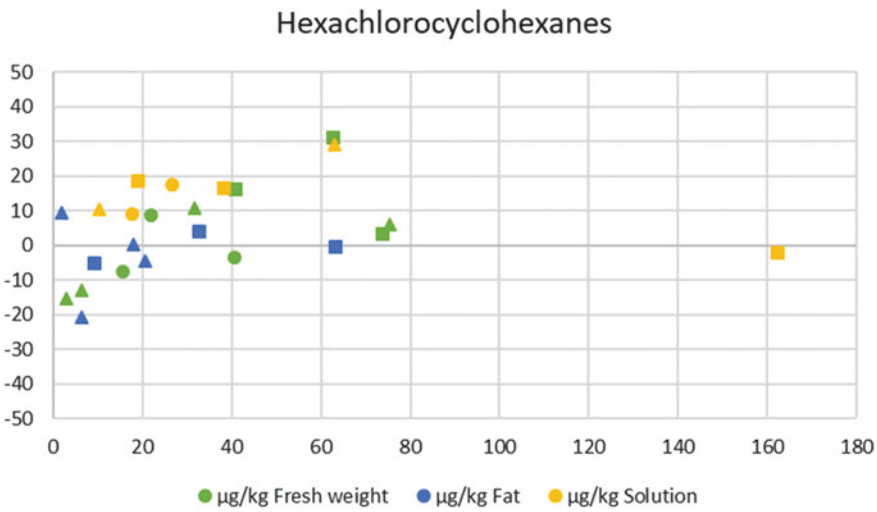

Fig. 26 Deviation (%) from the assigned values of 24 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for hexachlorocyclohexanes in proficiency tests performed between 2000 and 2019 (α-HCH results visualized as dots, β-HCH results as triangles, γ-HCH results as squares)

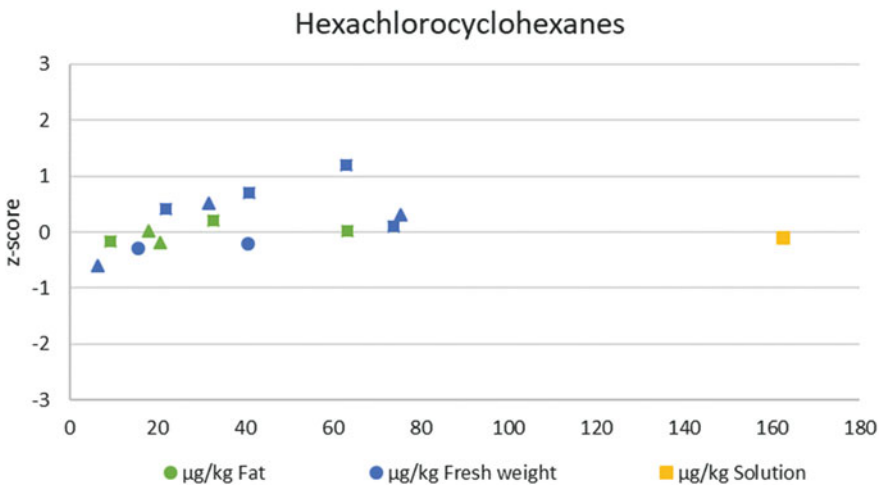

Fig. 27 Z-score of 15 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH) for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (α-HCH results visualized as dots, β-HCH results as triangles, γ-HCH results as squares)

4.3.8 Heptachlorepoxide

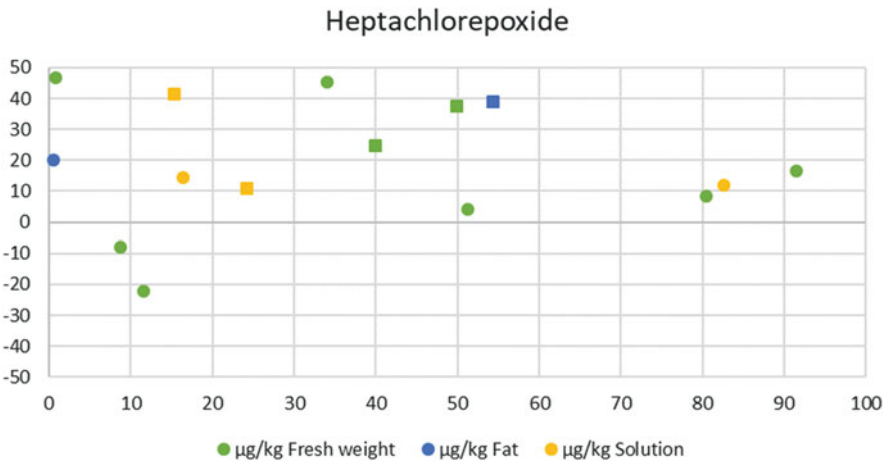

Fig. 28 Deviation (%) from the assigned values of 15 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for heptachlorepoxidee in proficiency tests performed between 2000 and 2019 (cis-heptachlorepoxide results visualized as dots, trans-heptachlorepoxide results as squares)

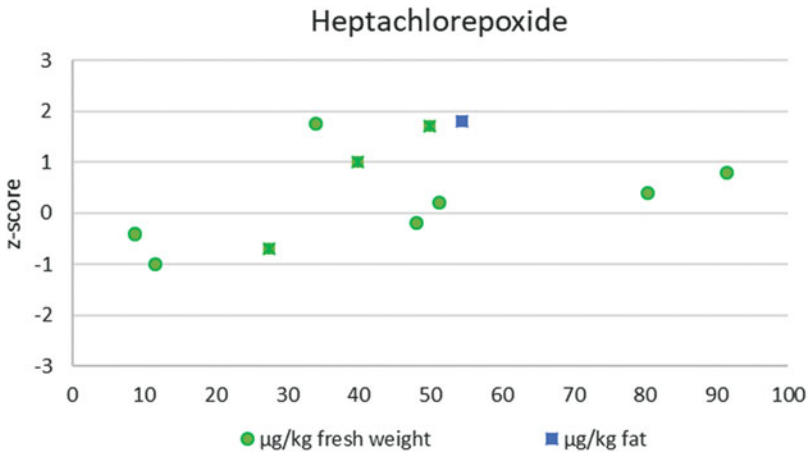

Fig. 29 Z-score of 11 results for heptachlorepoxidee for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (cis-heptachlorepoxide results visualized as dots, trans-heptachlorepoxide results as squares)

4.3.9 Mirex

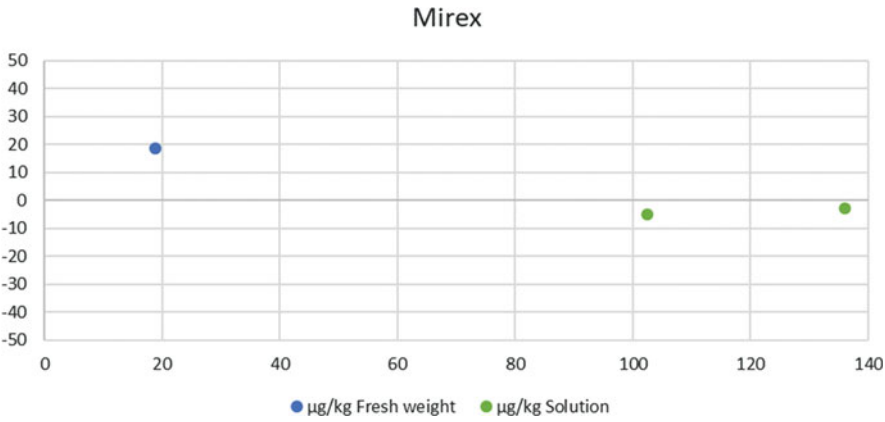

Fig. 30 Deviation (%) from the assigned values of 3 results (µg/kg fresh weight or µg/kg solution) for mirex in proficiency tests performed between 2016 and 2019 (no z-scores for these PTs)

4.3.10 Toxaphene

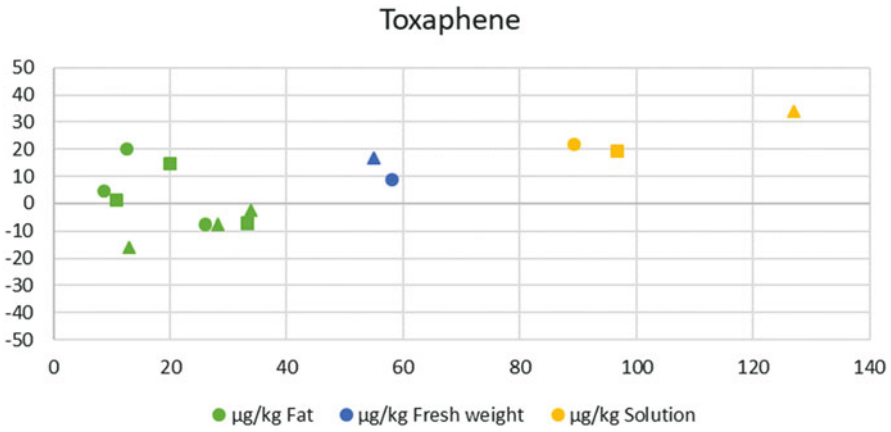

Fig. 31 Deviation (%) from the assigned values of 14 results (µg/kg fresh weight, µg/kg lipid or µg/kg solution) for toxaphene in proficiency tests performed between 1994 and 2019 (Parlar 26 results visualized as dots, Parlar 50 results as triangles and Parlar 60 results as squares)

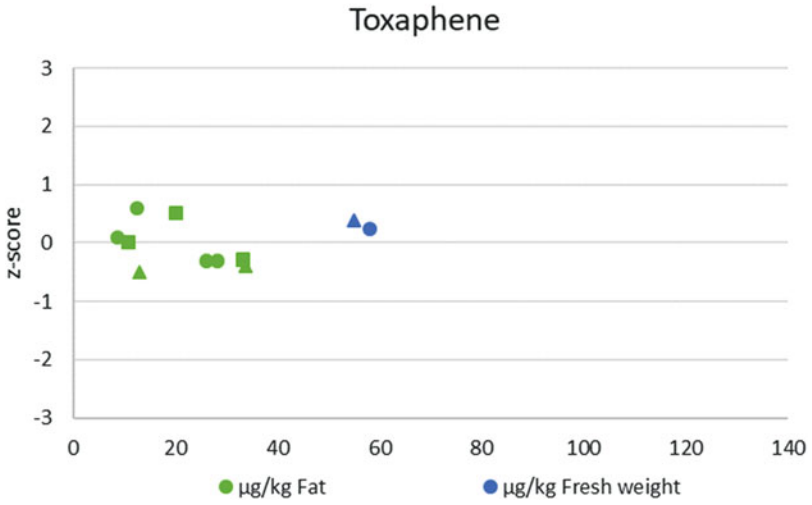

Fig. 32 Z-score of 11 results for toxaphene (Parlar 26, Parlar 50 and Parlar 60) for deviation from the assigned values of proficiency tests performed between 2000 and 2017 (Parlar 26 results visualized as dots, Parlar 50 results as triangles and Parlar 60 results as squares)

4.3.11 Average Deviation

A summary of the average deviation CVs (%) of all results from assigned values of proficiency tests performed between 1999 and 2019 regardless of the required reporting data (on lipid, fresh weight or volume basis) is shown in Table 6. No evaluation was possible for many individual parameters (n.a.) because the provider did not provide an assigned value or there was no offer. As result, based on the assigned values of proficiency tests, the methods achieved on average a long-term trueness over the 2000–2019 period calculated as deviation from the assigned value of 17.5%; 90% of the results differed by less than 27% from the assigned value.

The z-score performance in 51 proficiency test samples (see Sect. 4.3 above) for the analytes chlordane (cis-chlordane, trans-chlordane, oxychlordane), DDT (p,p'-DDT, p,p'-DDE, p,p'DDD), endosulfan (alpha-endosulfan, beta-endosulfan, endosulfan sulfate), endrin, hexachlorobenzene, hexachlorocyclohexanes (alpha-HCH, beta-HCH, gamma-HCH), heptachlor (heptachlor, cis-heptachlorepoxidee, trans-heptachlorepoxidee), toxaphene (Parlar 26, Parlar 50 and Parlar 62) and chlordecone is illustrated in Fig. 33. In accordance with the ISO 17043, z-scores of $|z| \leq 2.0$ indicates a "satisfactory" performance and generates no signal, z-scores between the range $2.0 < |z| < 3.0$ indicates a "questionable" performance and generates a warning signal and z-scores above $|z| \geq 3.0$ indicates a "unsatisfactory" performance and generates an action signal. In accordance with the ISO 17025, root cause analysis and corrective measures were initiated when z-scores were above $|z| \geq 2.0$. As conclusion, 92% of the z-score values were satisfactory.

4.4 Accreditation

In 1993, quality standards were introduced for laboratories entrusted by the Member States of the European Economic Community with the official control of foodstuffs: Laboratories had to comply with the general criteria for the operation of testing laboratories laid down in European Standard EN 45001 supplemented by standard operating procedures and the random audit of their compliance by quality assurance personnel not later than November 1998 (Council Directive 93/99/EEC). In a revision of the regulations on official controls in 2004, it was stipulated that laboratories that were designated for official control should operate and be assessed and accredited in accordance with the European Standard EN ISO/IEC 17025 (European Norm (EN) 17025) on "General requirements for the competence of testing and calibration laboratories" (EU Regulation 882/2004, succeeded by Regulation (EU) 2017/625). Therefore, the CVUA Freiburg was accredited in 1998 and has since been re-accredited continuously.

As a result, all analyses performed by CVUA Freiburg for the WHO/UNEP-coordinated exposure studies for the period 2000–2019 followed the strict rules of the accreditation system and the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025.

Table 6 Average absolute deviation (%) and median absolute deviation (%) for 14 parameters with 23 analytes

Analyte	Average absolute deviation (%)	Median absolute deviation (%)
Aldrin	n.a.	n.a.
Chlordane		
• Oxychlordane	11.8	10.5
• α-chlordane	18.1	10.0
• γ-chlordane	12.4	11.3
DDT		
• p,p'-DDE	12.2	8.4
• p,p'-DDT	18.7	18.3
• o,p'-DDT	22.4	22.4
• p,p'-DDD	21.2	9.5
Dicofol	n.a.	n.a.
Dieldrin	23.1	24.3
Endosulfan		
• Endosulfan-sulfate	25.8	23.1
• α-endosulfan	33.2	26.8
• β-endosulfan	27.8	32.5
Endrin	16.6	6.5
Heptachlorepoxide		
• cis-heptachlorepoxidee	19.8	15.6
• trans-heptachlorepoxidee	30.5	37.2
Hexachlorobenzene	16.7	13.9
Hexachlorobutadiene	n.a.	n.a.
Hexachlorocyclohexanes		
• α–HCH	9.4	8.4
• β-HCH	11.9	10.5
• γ-HCH	10.5	7.0
• δ-HCH	n.a.	n.a.
Mirex	8.9	5.1
Pentachlorophenol	12.5	12.5
Toxaphene		
• Parlar 26	12.5	8.6
• Parlar 50	15.3	16.0
• Parlar 62	10.4	10.8
Summary		
Min	8.9	5.1
Median	16.6	11.3
Average	17.5	15.2
90% Percentile	27.4	26.3
95% Percentile	30.1	31.7
Max	33.2	37.2
n.a.	No evaluation possible	

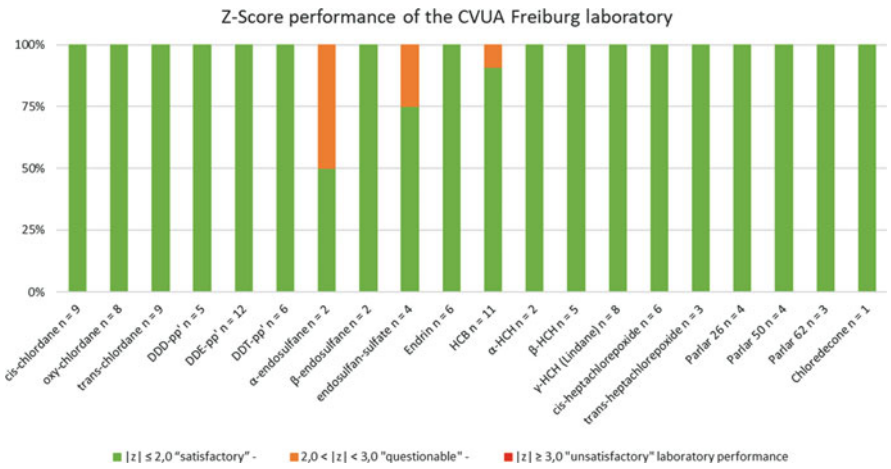

Fig. 33 Z-Score performance

5 Conclusion

The data of a comprehensive quality control gathered over two decades prove that the analytical methods applied for all samples of the WHO/UNEP-coordinated exposure studies performed between 2000 and 2019 met the requirements for acceptance of results as requested by the analytical criteria for analyses of organochlorine pesticides in food by EU legislation.

As a result, it was made sure that any differences in levels in this wide span of altogether 20 years (when the samples of the studies reported here were collected), e.g. lower levels found in later years, were not the result of an analytical variation but the result of decreasing temporal trends. As conclusion of various approaches of analytical quality control, for the 2000–2019 period the long-term trueness was on average in the range 85%–95% with a precision on average below 15%.

Acknowledgements The authors thank the team at CVUA Freiburg for performance of the pesticide analyses, especially Ina Wegert, Monika Golz, Manfred Grosse, Ralf Brandstetter, Sabine Walter, and Heike Müller for the analysis and for GC measurements. Biljana Trajkovska is acknowledged for her scientific support.

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Analysis and Quality Control of WHO- and UNEP-Coordinated Human Milk Studies 2000–2019: Polybrominated Diphenyl Ethers, Hexabromocyclododecanes, Chlorinated Paraffins and Polychlorinated Naphthalenes

Alexander Schächtele, Björn Hardebusch, Kerstin Krätschmer, Karin Tschiggfrei, Theresa Zwickel, and Rainer Malisch

Abstract

Four different analytical methods were used for the determination of (1) polybrominated diphenyl ethers (PBDE), (2) hexabromocyclododecanes (HBCDD), (3) chlorinated paraffins (CP) and (4) polychlorinated naphthalenes (PCN) in human milk samples of the WHO/UNEP-coordinated exposure studies. As a laboratory accredited according to EN ISO/IEC 17025, a comprehensive quality control program was applied to assure the reliability of results. This included procedural blanks, the use of numerous quality control samples as in-house reference materials and the participation in proficiency tests (PTs). Trueness was estimated from the PT samples using the assigned values.

The mean absolute deviation of the sum parameters $\sum PBDE_6$ and $\sum PBDE_7$ from the assigned values of 53 PT samples analysed between 2006 and 2021 was 12% and 14%, respectively.

For α-HBCDD as the most abundant diastereomer and the sum of α-, β- and γ-HBCDD, deviations of the reported value from the assigned value of the proficiency tests (31 samples, analysed between 2007 and 2021) were in most cases below 40% over a large concentration range, e.g., for α-HBCDD, between 0.0084 and 19 ng/g fw. For concentrations above 0.5 ng/g lipid, the deviation was in the range of approximately 0–30%.

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_6

For short-chain and medium-chain CP (SCCP and MCCP) all z-scores achieved in interlaboratory comparisons during 2017–2020 were within ±2 z and therefore satisfactory (13 PT samples were analysed for ΣCP, ΣSCCP and ΣMCCP using the GC-ECNI-Orbitrap-HRMS method, eight results achieved for ΣCP using the GC-EI-MS/MS method).

Due to the lack of available proficiency tests for PCN at the time of measuring the human milk samples of the 2016–2019 period, an external validation for control of the trueness was performed through an interlaboratory comparison with an independent laboratory. The deviation of the ΣPCN_{13} in five test samples between the external laboratory and CVUA Freiburg was in the range from 3 to 20%. At a later stage (in 2021), the laboratory participated successfully in the first interlaboratory comparison study on PCN congeners in cod liver oil. The z-scores for seven congeners and two sum parameters were within ±2 z and therefore satisfactory. Also, the results for other of the altogether 26 PCN congeners were in accordance with the median values reported by all participants.

As a result, the determination of PBDE, HBCDD, CP and PCN in human milk samples of the WHO/UNEP-coordinated exposure studies followed the strict rules of the accreditation system and the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025.

Keywords

Analysis · Polybrominated diphenyl ether (PBDE) · Hexabromocyclododecane (HBCDD) · Chlorinated paraffins (CP) · Short-chain chlorinated paraffins (SCCP) · Medium-chain chlorinated paraffins (MCCP) · Polychlorinated naphthalene (PCN) · Quality control · Trueness · Long-term precision · Human milk · Global WHO/UNEP studies · Human exposure

1 Introduction

This compendium "Persistent Organic Pollutants in Human Milk" comprises a series of articles on human milk surveys structured in five parts. Part I, the introduction, provides an overview of the World Health Organization (WHO) and the United Nations Environment Programme (UNEP)-coordinated exposure studies on persistent organic pollutants (POPs) in human milk and their link to the Stockholm Convention, including protocols for the collection of samples and an overview on the participating countries with respect to regional distribution and temporal differentiation (Malisch et al. 2023a). It also includes a review on human milk surveys on POPs (Fürst 2023) and a review on the Stockholm Convention and its implementation by regional and global monitoring reports (Šebková 2023). Part II presents analytical methods and quality control. In Part III, the findings between 2000 and 2019 are presented in various publications, some of which are relevant to this article: (1) polybrominated substances (Schächtele et al. 2023), (2) chlorinated paraffins (CP) (Krätschmer et al. 2023) and (3) polychlorinated naphthalenes (Tschiggfrei

et al. 2023). Part IV comprises assessments of time trends and of possible health risks for the breastfed infant that arise from dioxin-like compounds, and Part V presents conclusions and key messages.

This chapter describes the analytical methods and quality control measures used for the determination of polybrominated diphenyl ethers (PBDE), hexabromocyclododecanes (HBCDD), chlorinated paraffins (CP; comprising short-chain chlorinated paraffins [SCCP] and medium-chain chlorinated paraffins [MCCP]) and polychlorinated naphthalenes (PCN) in human milk samples obtained from the WHO/UNEP-coordinated exposure studies performed between 2000 and 2019.

2 Analytical Criteria

Accuracy depends on systematic errors and random components. "Trueness" (closeness of the agreement between the expectation of a test result or a measurement result and a true value (ISO 3534-2:2006, /24/)) and "Precision" (closeness of the agreement between independent test/measurement results obtained under stipulated conditions (ISO 3534-2:2006, /24/)) are used to describe accuracy and are therefore important criteria for the assessment of reliability of analytical methods (Eppe et al. 2017).

To provide reliable monitoring information for the Parties to the Stockholm Convention, the guidance document for the Global Monitoring Plan (GMP) proposed that a quantified objective for temporal studies should be stated, e.g. "to detect a 50% decrease in the levels of POPs within a 10-year period" (UNEP 2013, 2019). The statistical model used in the "Bi-ennial Global Interlaboratory Assessments on Persistent Organic Pollutants" is based on a target error of 25% to assess the performance of each laboratory for each analyte in each matrix. The analyte groups in the third round (2016/2017) and fourth round (2018/2019) of these assessments included organochlorine pesticides, polychlorinated biphenyls, dioxin-like POPs, PBDE, hexabromobiphenyl (only in the 2016/2017 round; however, without an assigned value for human milk), toxaphene, HBCDD (without assigned values for human milk in the 2016/2017 and 2018/2019 rounds) and perfluorinated alkyl substances, but not CP and PCN (UNEP 2017, 2021).

As of 2022, there were no analytical criteria defined for PBDE, HBCDD, PCN and short-, medium- or long-chain chlorinated paraffins (SCCP, MCCP, LCCP, respectively) in the European Union. The European Union Reference Laboratory (EURL) for halogenated POPs in feed and food published a general guidance document on SCCP and MCCP analysis in food samples (EURL for Halogenated POPs in Feed and Food 2021, with the annex describing analytical criteria under review). Interlaboratory studies and proficiency tests in that field commonly use a variation of ±25% as target standard deviation, leading to results within a variation of ± 50% being acceptable (Krätschmer and Schächtele 2019). Additionally, the EURL POPs published a guidance document on the determination of brominated

contaminants, initially specifying analytical criteria for PBDE and HBCDD (EURL for Halogenated POPs in Feed and Food 2022a; Fernandes et al. 2022a).

3 Polybrominated Diphenyl Ethers (PBDE)

3.1 Analytes

For PBDE analysis, six prevalent analytes (comprising seven congeners) were recommended by the GMP Guidance Document for the groups from tetra-BDE to hepta-BDE (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-175/183 [co-eluting]) (UNEP 2013, 2019). The sum of these six PBDE analytes was used as the most important summarizing parameter until 2017, when BDE-209 was added (UNEP 2019). However, questions were raised whether BDE-175 is an important enough component of commercial octabromodiphenyl ether mixtures to be listed in Annex A of the Stockholm Convention. Since BDE-175 and BDE-183 co-elute on common HRGC columns, the presence of BDE-175 as an important component in technical octa-BDE mixtures has not been illustrated. The successful HRGC/LRMS separation of a 1:1 mixture of BDE-175 and BDE-183, as well as ^{1}H NMR analysis of technical material, has allowed to confirm that this congener is not present in technical products (e.g. Great Lakes DE-79™) in quantifiable amounts (Konstantinov et al. 2011). Therefore, the "Guidance document on the determination of organobromine contaminants for analytical parameters in food and feed" recommends 9 PBDE congeners as analytes of interest: seven congeners covered by the GMP Guidance Document (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209) and in addition BDE-28 and BDE-49 (EURL for Halogenated POPs in Feed and Food 2022a).

For the human milk samples of the period 2016–2019, in addition to the congeners recommended by the GMP guidance document (except BDE-175), 18 congeners were included to cover a broader range (BDE-15, BDE-17, BDE-28, BDE-49, BDE-66, BDE-75; BDE-77; BDE-85; BDE-119; BDE-126; BDE-138; BDE-190; BDE-196; BDE-197; BDE-203; BDE-206; BDE-207; BDE-208). Whereas BDE-28 and BDE-49 were included in EURL proficiency test, the other additional congeners and BDE-175 were not included in any proficiency tests, and therefore are currently not validated with the same degree of quality control as the nine analytes recommended by the EURL Guidance Document. However, results can give a first provisional indication whether other congeners might be of interest in addition to the congeners recommended by the GMP guidance document. Table 19 (in the appendix) lists these 25 PBDE congeners and the 13 $^{13}C_{12}$-labelled internal standards.

3.2 Extraction

The lipid portion of the milk is extracted as a first step, and the two procedures used for this are described below. $^{13}C_{12}$-labelled surrogates (or other $^{13}C_{12}$-labelled

congeners) of many of the analytes are added to an aliquot of the extracted lipids and serve as internal standards, for more accurate quantitation and also for the calculation of recoveries.

3.2.1 Separation of the Cream (Lipid) Layer by Centrifugation

For extraction of the lipids, approx. 200 g of human milk sample was centrifuged in stainless steel centrifuge tubes for 10 min at approx. 3000 rpm at approx. 4 °C. The supernatant cream layer was transferred into a glass beaker. Sodium sulphate was added to the cream until the ground material was powdery when stirred. This powder was extracted 3–4 times with n-hexane by stirring well with a glass rod and filtering the extract. The solvent was rotary evaporated to give the extracted lipids. The lipids were dried using a nitrogen stream on a sand bath at approx. 80 °C for 30 min and in a drying oven at 103 °C for 30 min.

3.2.2 Twisselmann Hot Extraction

Before extraction, the sample was freeze-dried or mixed with a drying agent (polyacrylate or sodium sulphate). A mixture of ethanol and toluene (7/3, v/v) was used for the Twisselmann extraction (permanent "hot extraction" at the boiling point of the solvent mixtures for six hours). After evaporation of the solvent, the raw extract was dissolved in *tert*-butyl methyl ether (t-BME) for separation of polar co-extractives and for lipid determination after evaporation of t-BME.

3.2.3 Addition of Internal Standards

To an aliquot of the lipid aliquot, the $^{13}C_{12}$-labelled standards listed in Table 19 were added.

3.3 Clean-up

Principle

The extracts obtained from the above procedures require further purification. Manual or automated purification procedures are based on similar principles that essentially separate PBDE from the lipids and other co-extracted interferences using adsorbents such as alumina, silica, Florisil (activated magnesium silicate) or techniques such as gel permeation chromatography (GPC).

For the analysis of human milk, two different procedures were applied. A partly automated method including gel permeation chromatography, a multi-layer silica column and a Florisil column was used for the determination of BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, whereas a fully automated method applying DEXTech Plus/Pure (LCTech, Obertaufkirchen, Germany) was used for these analytes including BDE-209 and the other specified BDE. Both methods include elements of the analytical procedures that are used for the determination of PCDD/PCDF and PCB and allow the simultaneous clean-up in different fractions, where required.

3.3.1 Partly Automated Clean-up Procedure

Gel permeation chromatography using Bio-Beads S-X3 (Bio-Rad, Hercules, CA, USA) was used to remove the lipid (in four runs with a maximum of 0.75 g fat each; 50 g Bio-Beads S-X3; eluent ethyl acetate/cyclohexane [1/1, v/v]). Small amounts of remaining lipid and oxidizable substances were removed using a mixed column loaded with layers of 1 g sulphuric acid (96%) impregnated silica gel and 1 g NaOH-impregnated silica gel (eluent: 20 mL heptane). A Florisil column (deactivated with 3% water) was used for further clean-up (elution of PBDE by heptane containing 0.2% of toluene; PBDE collected as the first fraction). (*If PCB and PCDD/PCDF are co-analysed, PCB co-elute with PBDE in this first fraction; PCDD/PCDF are eluted by toluene in a second fraction*).

3.3.2 Fully Automated Clean-up Procedure

The fat extract was dissolved in 1 mL of acetone and 5 mL of cyclohexane and injected into the purification system of the fully automated clean-up system (DEXTech Plus/Pure) with a 15 mL sample loop). The method for determination of PBDE uses the same method columns and eluents as used for the determination of PCDD/PCDF and PCB, thus, the clean-up for these three groups of analytes can be performed simultaneously. The method combines three columns: (1) a multi-layer silica sulphuric acid column (column 1); (2) an alumina column (column 2) and (3) a carbon column (column 3). Pre-packed columns were provided ready-to-use by the supplier. The sample extract was loaded with hexane first onto the multi-layer silica column, then transferred with hexane onto the alumina column. PBDE were eluted from the alumina column by a mixture of n-hexane and dichloromethane (1/1, v/v 50:50%) onto the carbon column. PBDE were not adsorbed by the carbon column (*in contrast to PCDD/PCDF and non-ortho PCB*) and collected as Fraction 1 (Table 1).

This fraction was concentrated to 3 mL followed by the addition of 10 µL of dodecane, then carefully evaporated to dryness under a gentle stream of nitrogen and finally reconstituted with 90 µL toluene containing the recovery (syringe) standards $^{13}C_{12}$-BDE-77 and $^{13}C_{12}$-BDE-206.

Table 1 Method parameters for the DexTech Plus/Pure system collecting PBDE in fraction 1 (*if required, ndl-PCB and mono-ortho-PCB may also be collected in fraction 1; non-ortho PCB and PCDD/PCDF, in fraction 2*)

Step	Solvent	Volume (mL)	Flow (mL/min)	Time (min)	Analytes
1. Load onto multi-layer column, purification on multi-layer column, elution to alumina column					
Solvent	Hexane	182	7	26	
2. Elution from alumina column onto carbon column and collection of PBDE fraction					
Fraction 1	DCM/ Hexane	36	3	12	**PBDE** *(ndl-PCB, mono-ortho-PCB)*
3. *Elution of PCDD/PCDF and non-ortho PCB from carbon column, if of interest*					
Fraction 2	Toluene	10	1	10	*(non-ortho-PCB, PCDD/ PCDF)*
N_2				3	

3.4 GC-HRMS Measurement

The measurement of the PBDE was carried out using a GC-HRMS system (MAT95XP and DFS, Thermo Fisher Scientific, Waltham, MA, USA) monitoring M^+ or M-2Br$^+$ clusters at a resolution of 10,000 (5% valley). 5 µL of the final extract was injected into a PTV inlet using the solvent vent mode. Analytes of interest were separated on a 15 m or 30 m Rtx-1614 column (Restek, Bellefonte, PA, USA) or a 30 m DB-5 MS column (Agilent Technologies, Santa Clara, CA, USA) (Fig. 1).

3.5 Quality Control

Human milk samples were received at the reference laboratory over 20 years in five rounds between 2000 and 2019, each covering approximately 4 years, and analysed for PBDE between 2005 and 2020, including determination of PBDE in left-over samples of the 2000–2004 period. In order to check the comparability of results, an internal quality control program was run covering procedural blank samples, various kinds of in-house reference samples and external quality control based on regular participation in interlaboratory studies and proficiency tests.

3.5.1 Procedural Blank Samples

For the six PBDE congeners BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183, 116 procedural blank samples were analysed between 2005 and 2021. These procedural blanks were included in all steps of the analytical method. Calculations for the limits of quantification (LOQs) were based on the use of an aliquot equivalent to 3 g lipid. In contrast to BDE-153, BDE-154 and BDE-183, the lower brominated congeners BDE-47 and BDE-99 were above the respective LOQs in more than half of the samples. Median procedural blank levels for the individual

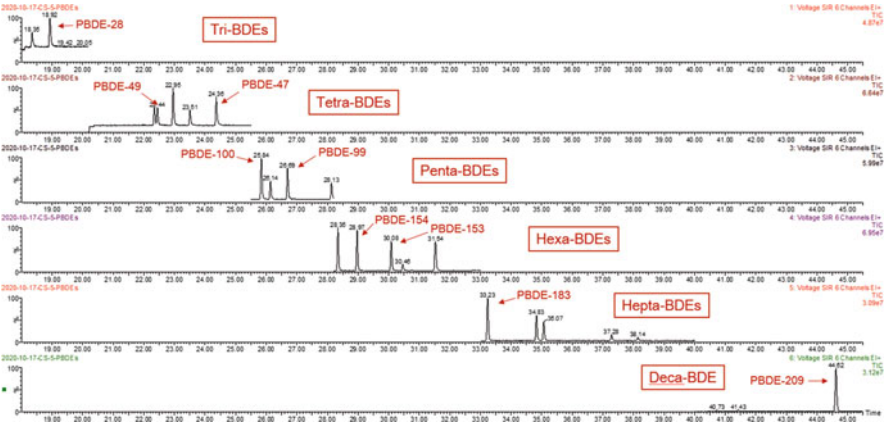

Fig. 1 Example chromatogram showing separation of PBDE congeners by GC-HRMS (30 m Rtx-1614; R = 10,000)

congeners were in most cases below the lowest levels found in the analysed human milk samples. In some cases, the maximum levels for procedural blanks were in the range of the minimum levels found in human milk samples for these congeners. In these cases, a contribution from the method background (as seen in the procedural blanks) to very low levels found in human milk cannot be excluded. However, the median concentrations of the individual BDE congeners in human milk were about two orders of magnitude higher than the median of the blank samples (range 34–650 times higher), the sum parameter $\sum PBDE_6$ by a factor of 140 (in comparison with the maximum found in the blank samples, by a factor of 8) (Table 2). The sum parameter was calculated as "upper-bound" (ub) result (with calculation of the contribution of not quantified congeners to the sum parameter as the limit of quantification).

BDE-209 was determined in human milk samples collected in the 2016–2019 period; these analyses were performed from 2019 to 2021. The BDE-209 results for procedural blanks analysed in the same batch with these human milk samples were in the range 0.04–0.07 ng/g lipid and therefore below the levels found in human milk. Results for the sum parameter $\sum PBDE_7$ (ub) for the procedural blank samples were in all cases below levels found in the human milk samples (median of $\sum PBDE_7$ in human milk by a factor of 30 higher than median of blank samples).

3.5.2 Quality Control Samples as In-House Reference Material

Six different quality control (QC) samples were used for monitoring of the precision of PBDE analysis between 2004 and 2021. Both naturally contaminated samples and samples spiked with native PBDE congeners were analysed. An overview of the different quality control samples, the number of replicate analyses and the results are given in Table 3. The concentration for the sum parameters $\sum PBDE_6$ and $\sum PBDE_7$ ranged between 0.1 and 9.2 ng/g lipid, and 0.2 and 9.3 ng/g lipid, respectively, and therefore covered most of the concentration range in human milk samples, except for very highly contaminated samples. The coefficients of variation (CVs) for the sum parameters $\sum PBDE_6$ and $\sum PBDE_7$ were in the acceptable range between 9 and 16%, and 6 and 34%, respectively.

Figure 2 gives an overview of the CVs of the individual congeners for the six different QC samples compared with the mean content of these congeners in the QC samples. For most of the congeners, CVs below 30% were observed and only in some cases did CVs exceed 40%.

In conclusion, these QC samples analysed between 2004 and 2021 show a good comparability of the results for sum parameters and in most cases also for individual congeners over this period of time.

3.5.3 Participation in Proficiency Tests

Between 2006 and 2021 CVUA Freiburg participated in numerous interlaboratory studies and proficiency tests generating results for individual congeners and sum parameters for up to 53 different food test samples. The concentration ranges of these proficiency test covered a very wide concentration range from the low pg/g fresh weight (fw) range to the high ng/g fw range. Many of these PT samples were fat or oil samples and in these cases the concentration reported on a fat basis equalled the

Table 2 Concentrations of six PBDE congeners and $\sum PBDE_6$ (ub) of reagent blank samples analysed together with human milk and fatty food samples between 2005 and 2021 and of BDE-209 and $\sum PBDE_7$ (ub) analysed between 2019 and 2021 together with the human milk samples of the 2016–2019 period; the concentration range found in human milk samples is included for comparison

	Blank samples		Blank samples			For comparison: human milk			
			Median	Mean	Maximum	Min	Median	Mean	Max
Parameter	No. of analyses	No. of results > LOQ	ng/g lipid	ng/g lipid	ng/g lipid	ng/g lipid	ng/g lipid	ng/g lipid	ng/g lipid
BDE-47	116	104	0.0051	0.0078	0.049	0.09	0.94	4.60	234
BDE-99	116	64	0.0029	0.0079	0.073	0.03	0.27	1.30	58.6
BDE-100	116	37	0.0018	0.0027	0.038	0.02	0.19	0.87	39.1
BDE-153	116	38	0.0006	0.0016	0.011	0.08	0.39	0.85	17.5
BDE-154	116	33	0.0007	0.0019	0.018	0.01	0.03	0.10	3.07
BDE-183	116	52	0.0014	0.0075	0.20	0.01	0.05	0.08	0.71
BDE-209	5	5	0.04	0.05	0.07	0.09	0.21	0.62	5.92
$\sum PBDE_6$ (ub)	116		0.013	0.029	0.24	0.28	1.79	7.99	352
$\sum PBDE_7$ (ub)	5		0.06	0.06	0.07	0.53	1.76	6.55	113

Table 3 Quality control samples analysed between 2004 and 2021 for PBDE and CVs of the sum parameters $\sum PBDE_6$ and $\sum PBDE_7$

No.	Matrix	No. of replicates	$\sum PBDE_6$ ng/g lipid	$\sum PBDE_7$ ng/g lipid	$\sum PBDE_6$ CV (%)	$\sum PBDE_7$ CV (%)
1	Mixed fat (2004–2016)	38	1.47	–	15	–
2	Mixed fat (2017–2021)	46	0.36	1.2	9	34
3	Milk fat (2019–2020)	49	0.12	0.16	10	14
4	Spiked vegetable oil (2020–2021)	15	0.21	1.1	13	7
5	Fish oil (2015–2021)	17	9.2	9.3	11	6
6	Fish oil (2019–2021)	34	7.2	7.4	16	16
	Min	15	0.12	0.16	9	6
	Max	49	9.2	9.3	16	34

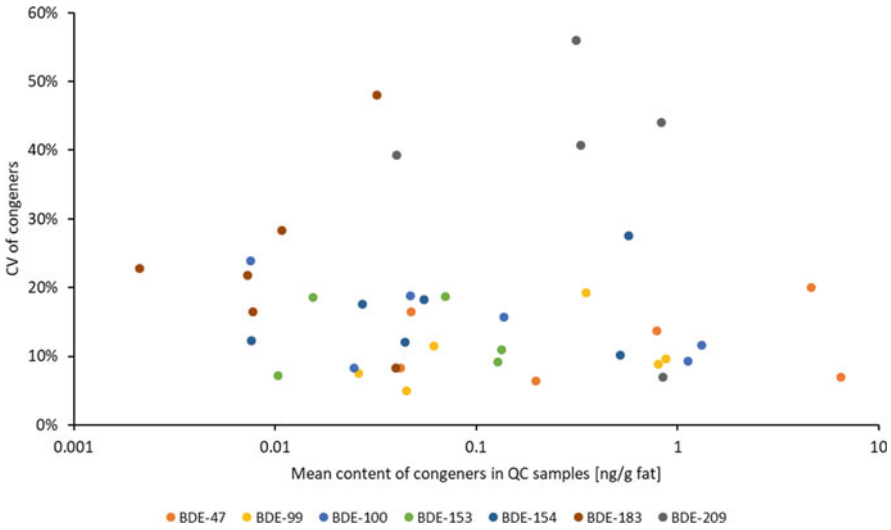

Fig. 2 Mean content of PBDE congeners in six quality control samples (ng/g lipid) plotted against coefficients of variation of these congeners

concentration on a fresh weight basis. An overview of the results is given in Table 4 showing the wide concentration range and the mean absolute deviation. Figure 3 illustrates the deviations of individual results from the assigned values, Fig. 4 the deviations of the sum parameters.

Table 4 Results of proficiency tests for PBDE congeners and sum parameters between 2006 and 2021

		Assigned value		
		Minimum	Maximum	Mean absolute deviation
Parameter	No. of results	ng/g fw	ng/g fw	(%)
BDE-47	53	0.00096	54	13
BDE-99	53	0.00089	27	18
BDE-100	53	0.00045	19	18
BDE-153	53	0.00042	5.5	13
BDE-154	53	0.00017	5.0	19
BDE-183	42	0.00018	2.3	37
BDE-209	9	0.0097	2.1	31
$\sum PBDE_6$	53	0.0029	98	12
$\sum PBDE_7$	9	0.013	7.4	14

Note that many of these PT samples were fat or oil samples and in these cases the concentration on fat basis equals the concentration on a fresh weight basis

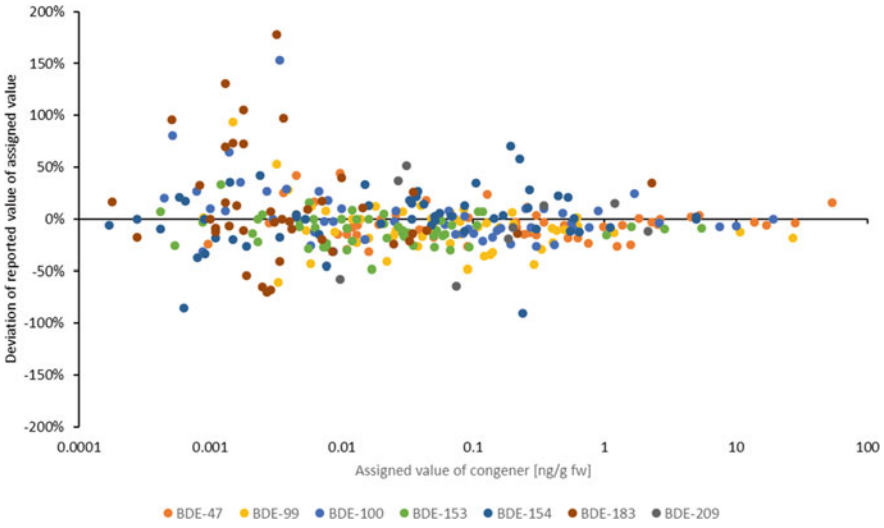

Fig. 3 Deviation of results reported for individual congeners of the assigned values for proficiency tests between 2006 and 2021 (Note that many of these PT samples were fat or oil samples and in these cases the concentration on fat basis equals the concentration on a fresh weight basis.)

The mean absolute deviation of the reported results of the individual congeners from the assigned values was in most cases below 20% with higher values above 30% for BDE-183 and BDE-209, the deviation of the sum parameters 12% for $\sum PBDE_6$ and 14% for $\sum PBDE_7$.

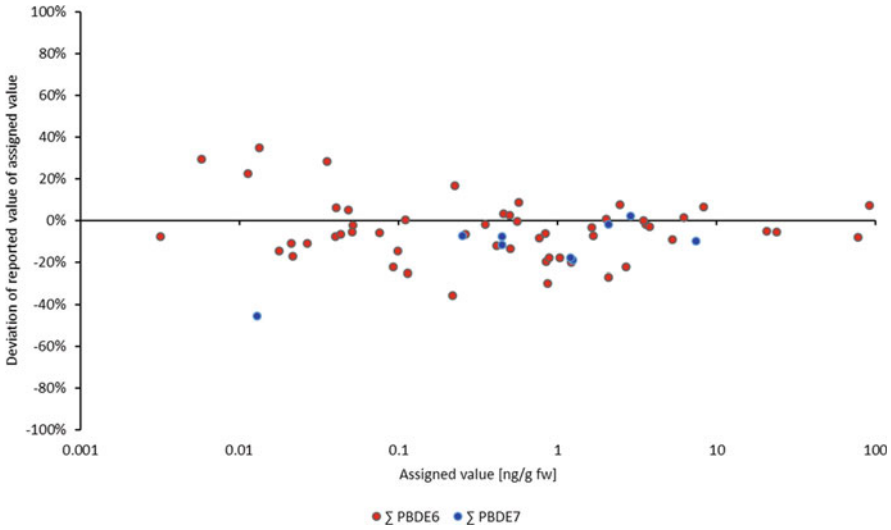

Fig. 4 Deviation of results reported for PBDE sum parameters from assigned values for proficiency tests between 2006 and 2021 (Note that many of these PT samples were fat or oil samples and in these cases the concentration on fat basis equals the concentration on a fresh weight basis.)

4 Hexabromocyclododecanes (HBCDD)

4.1 Analytical Procedure and Analytes

For determination of HBCDD, the extraction and clean-up steps of a method based on the European Norm 1528 for determination of residues of nonpolar organochlorine pesticides and contaminants were used, which is also part of the official collection of test methods in Germany (European Norm [EN] 1528; German Standard § 64 LFGB). It is based on a modular structure and covers a wide range of analyte-matrix combinations (suitable for food of animal origin and human milk).

After centrifugation at 3000 rpm for 10 min and separation of the cream, lipids were extracted with nonpolar solvents. The supernatant cream layer was transferred into a glass beaker. Sodium sulphate was added to the cream until the ground material was powdery when stirred. This powder was extracted 3–4 times with *n*-hexane by stirring well with a glass rod and filtering the extract. The solvent was evaporated to give the extracted lipids.

As internal standards, TCB (2,4,5′-trichlorobiphenyl), triphenylphosphate and PCB 209 (decachlorobiphenyl) were used. After addition of the internal standards, up to 0.5 g lipids was separated by gel chromatography using polystyrene gel (Bio-Beads S-X3; length 740 mm, 20 mm i.d., filling level 500–500 mm; cyclohexane/ethyl acetate mixture [1:1] as eluent). After concentration to about 2–3 mL volume, the eluate was further concentrated with isooctane added and evaporated

to about 1 mL. Ethyl acetate had to be completely removed, otherwise isooctane had to be added again and concentrated.

Chromatography on a small column of partially deactivated silica gel was performed as the final clean-up step. The silica gel (70–230 mesh) was heated overnight at 130 °C and allowed to cool in a desiccator. After adding 1.5% of water, it was shaken for 30 min and then stored in a tightly sealed container. The chromatographic tube was packed with 1 g of deactivated silica gel. After pre-washing the column with 2 × 5 mL hexane, the isooctane solution was loaded onto the silica column and the analytes of interest were eluted by a total volume of 10 mL toluene.

The eluate of this fraction was evaporated to a small volume (no complete dryness) and immediately taken up with 0.1 mL methanol in the intended final volume for determination by HPLS-MS/MS (Agilent TQ 6410, Agilent Technologies, Santa Clara, CA, USA) using a C18 column (50 × 2.1 mm, 1.8 μm ID, Agilent Technologies, Santa Clara, CA, USA).

4.2 Quality Control

4.2.1 Procedural Blank Samples

No α-, β- and γ-HBCDD concentrations above the limit of quantification were found in procedural blank samples (LOQ for 90% of the procedural blank samples: <0.1 ng/g lipid; max 0.5 ng/g lipid).

4.2.2 Quality Control Samples as In-House Reference Material

A fat sample spiked with α-, β- and γ-HBCDD at 5 ng/g lipid was used as an in-house reference material for quality control. Coefficients of variations for individual diastereomers and for the sum parameter were in an acceptable range between 15 and 17% (Table 5).

4.2.3 Participation in Proficiency Tests

The samples analysed for proficiency tests between 2007 and 2021 covered a wide concentration range between below 0.01 ng/g and above 10 ng/g fresh weight. Many of these PT samples were fat or oils and in these cases the concentration on fat basis equals the concentration on fresh weight basis. This range covers the concentrations

Table 5 HBCDD concentrations and precision (CV) determined in 106 quality control samples (spiked fat, 5 ng/g lipid) used as in-house reference materials

		α-HBCDD		β-HBCDD		γ-HBCDD		Sum α-, β-, γ-HBCDD	
Matrix	No. of replicates	ng/g lipid	CV (%)	ng/g lipid	CV (%)	ng/g lipid	CV (%)	ng/g lipid	CV (%)
Lard (2014–2020)	106	5.6	17	4.9	16	5.0	16	15.5	15

found in human milk samples: The α-HBCDD levels of 102 pooled samples from 72 countries collected between 2006 and 2019 ranged between <0.1 ng/g lipid and 15 ng/g lipid (median: 0.5 ng/g lipid; 90% of all results were below 2 ng/g lipid). In nearly all samples, β-HBCDD and γ-HBCDD occurred below or around the limit of quantification (Schächtele et al. 2023).

The deviations of the reported value from the assigned value of the proficiency tests for α-HBCDD as most abundant diastereomer and the sum of α-, β- and γ-HBCDD were in the range of approximately 0–30% for concentrations above 0.5 ng/g fw respectively lipid. The mean value of the absolute deviation was below 40% for all diastereomers and the sum parameter over the whole concentration range, e.g. for α-HBCDD the value was between 0.0084 ng/g fw and 19 ng/g fw (in this case, equal to 19 ng/g lipid) (Table 6, Fig. 5).

Table 6 Results of proficiency tests performed between 2007 and 2021 for HBCDD congeners and the sum parameter between 2007 and 2021

		Assigned value		Mean absolute deviation
		Minimum	Maximum	
Parameter	No. of results	ng/g fw	ng/g fw	(%)
α-HBCDD	31	0.0084	19	24
β-HBCDD	11	0.0086	0.40	28
γ-HBCDD	18	0.0048	0.97	37
Sum α-, β-, γ-HBCDD	26	0.021	20	27

Note that many of these PT samples were fat or oil samples and in these cases the concentration on a fat basis equals the concentration on a fresh weight basis

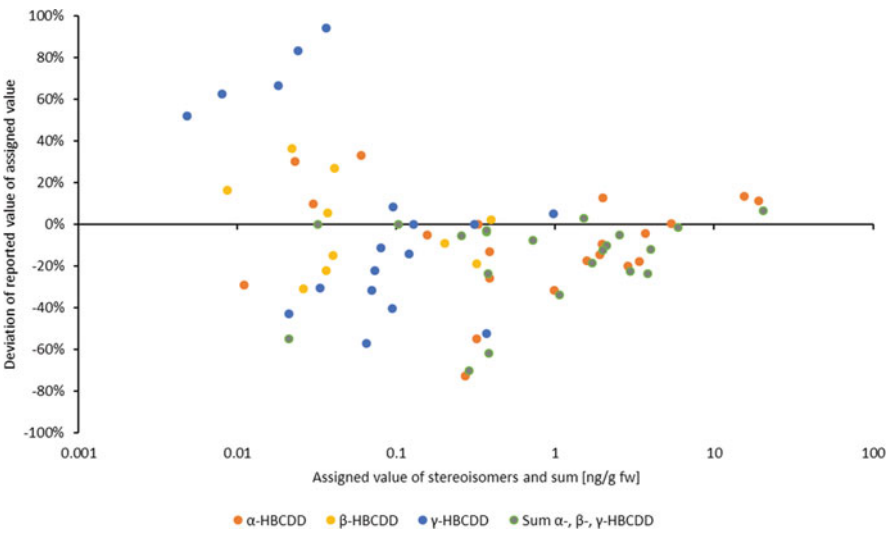

Fig. 5 Deviation of results reported for HBCDD from assigned values for proficiency tests between 2007 and 2021 (Note that many of these PT samples were fat or oil samples and in these cases the concentration on a fat basis equals the concentration on a fresh weight basis.)

5 Chlorinated Paraffins (CP)

The method for routine analysis of chlorinated paraffins in food and human milk was developed and validated as part of a doctoral thesis (Krätschmer 2022).

5.1 Analytical Procedure and Analytes

5.1.1 Sample Preparation

Sample preparation of the frozen human milk sample was performed as described elsewhere (Krätschmer et al. 2018, 2019, 2021). In brief, 50 g of warmed, homogenized samples were filled into baked-out glass centrifuge tubes. Cooled centrifugation (4 °C, 3000 rpm, 10 min) was used to separate the cream which was then removed from the hydrogenous phase. The cream was fortified with the recovery standard ($^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane, Cambridge Isotope Laboratories, Tewksbury, Ma, USA) and dried by grinding with sodium sulphate until a powdery consistency was reached. Manual cold extraction with dichloromethane/n-hexane (1:1, v/v) was performed in triplicate and the decanted, filtered solvent evaporated to dryness using a rotary evaporator.

Further sample clean-up was performed using open column chromatography (Fig. 6). In a first step, the lipids were hydrolysed on an acidified silica column. The resulting extract was fractionated on a Florisil® column (magnesium silicate primed with 1.5% water) eluted with 75 mL n-hexane followed by 60 mL dichloromethane. The latter fraction contained the CP and was concentrated, initially using a rotary evaporator followed by a gentle nitrogen stream before the addition of

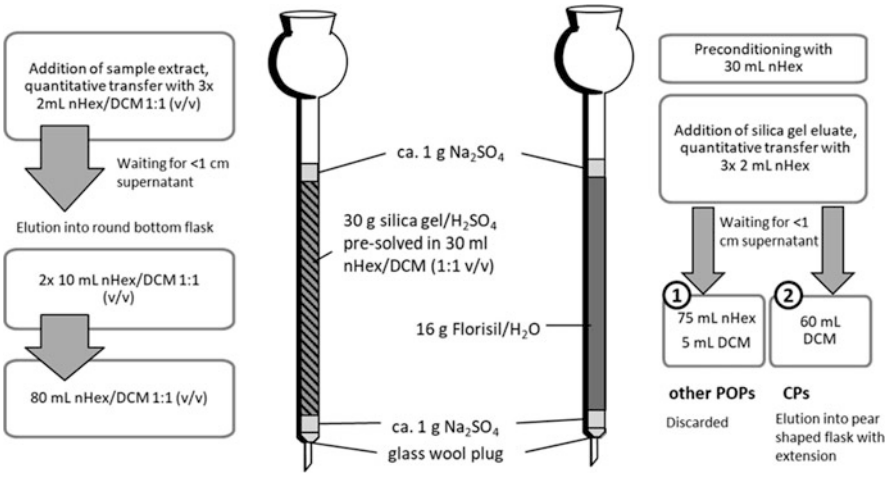

Fig. 6 Description and elution protocol of the acidified silica column (left) and Florisil column (right) used for sample clean-up

the syringe standard ε-hexachlorocyclohexane (ε-HCH, Dr. Ehrenstorfer, Augsburg, Germany).

Each sample batch additionally included a procedural blank (sodium sulphate prepared like a sample) as well as different quality control (QC) samples which consisted of raw cow's milk with and without fortification with SCCP and MCCP standards at different levels. For other sample series, QC samples originating from interlaboratory studies (e.g. fortified coconut fat, fortified lard) or prepared from market food (e.g. extra virgin olive oil) were used.

5.1.2 Analysis

Unlike all the other POPs analysed in these samples, CP are not separable by chromatographic methods (Fig. 7). Accordingly, standard quantification procedures using response factors of quantification standards of known concentration are not applicable here. Over the last decade, a multitude of quantification methods both differing in instrumentation used (e.g. liquid or gas chromatography, tandem gas chromatography, direct injection combined with low- or high-resolution mass spectrometry or tandem mass spectrometry) and data treatment strategies (e.g. peak deconvolution, different forms of calibration, direct application of pre-modelled response factors) have been published in the literature (van Mourik et al. 2015; Yuan et al. 2019; Fernandes et al. 2022b).

Although many of these approaches could be proven as comparable during international interlaboratory studies (van Mourik et al. 2018; Krätschmer and Schächtele 2019), the true concentration of CP in any given sample remains unknown. Another major difficulty is the poor availability of suitable quantification standards (Schinkel et al. 2018; Fernandes et al. 2022c), a situation that has shown some improvement in the last year or so. Given the major developments in this field of analysis in recent years, the method applied to human milk samples at CVUA Freiburg has also changed over the years. Therefore, both the method applied until 2016 and the current method will be briefly described in the following sections.

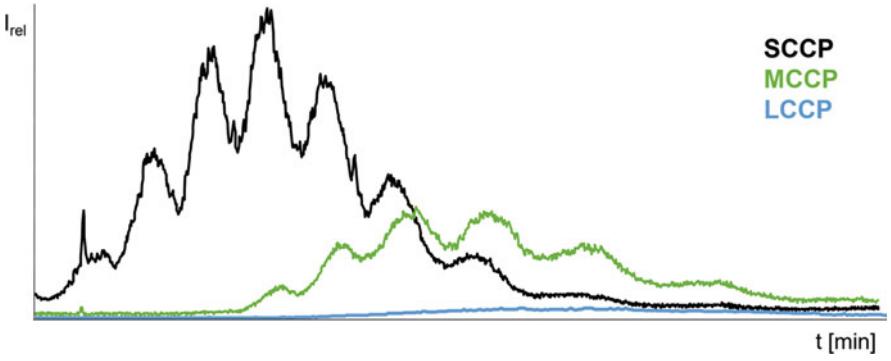

Fig. 7 Total ion chromatograms on a GC-ECNI-Orbitrap-HRMS instrument of an SCCP, MCCP and LCCP commercial quantification standard. LCCP are almost impossible to quantify using gas chromatography due to low volatility

Semi-quantitative Analysis (GC-EI-MS/MS)

Until 2016, human milk samples were analysed by GC-EI-MS/MS (Reth et al. 2005). This allowed for a reliable and comparable estimation of the total CP amount in the sample, but not for a distinction between SCCP and MCCP. The following parameters, as also shown in the EURL POPs Guidance Document on the Analysis of CP in Food, were applied (EURL for Halogenated POPs in Feed and Food 2021).

Electron ionization (EI) is a hard ionization method, i.e. it tends to create many small fragment ions. As CP have similar structures, this fragmentation is non-specific and reduces information of the parent molecules. Tandem mass spectrometry at least allows for the identification of three mass transitions that are specific for all CP and allows for separation from other organochlorine contaminants.

Each of the three mass transitions shown in Table 7 may be used to provide a semi-quantitative estimate of the total CP amount in a sample. As partial characterization, the results of all three transitions, quantified via (a) SCCP and (b) MCCP calibration solutions, may be reported as intermediate results. However, the end

Table 7 Method parameters for CP determination using GC-EI-MS/MS

Method parameters: GC-EI-MS/MS (Reth et al. 2005; Krätschmer et al. 2018)			
Instrument	7890A GC interfaced with a 7000B QQQ MS triple quadrupole system (Agilent Technologies, Santa Clara, CA, USA)		
Column	15 m length × 0.25 mm i.d., 0.25 µm film thickness HP5-MSUI fused silica capillary column (Agilent Technologies, Santa Clara, CA, USA) Transfer line temperature: 280 °C		
GC oven programme	60 °C (2 min), increase at 50 °C/min to 300 °C (5 min) Total time 11.8 min		
Injector	Compressed air cooled PTV injector (Gerstel, Mülheim a. d. Ruhr, Germany) in solvent vent mode (4.8 psi until 0.03 min) 5 µL sample is injected at 70 °C (holding time 0.13 min) and the injector then heated at a rate of 720 °C/min to 300 °C (holding time 5 min)		
Detection	EI-MS/MS (MRM mode), collision gas: Nitrogen Source electron energy 70 eV Mass transitions:		
	Target analyte	**Mass transition [*m/z*]**	**CE [eV]**
	CPs Trans 1	102 $[C_5H_7Cl]^+ \rightarrow$ 67 $[C_5H_7]^+$	10
	CPs Trans 2	102 $[C_5H_7Cl]^+ \rightarrow$65 $[C_5H_5]^+$	20
	CPs Trans 3	91 $[C_7H_7]^+ \rightarrow$53 $[C_4H_5]^+$	10
	$^{13}C_{10}$-hexachlorodecane Trans 1	142→78	11
	$^{13}C_{10}$-hexachlorodecane Trans 2	142→106	5
	ε-HCH Trans 1	180.8→144.9	15
	ε-HCH Trans 2	180.8→109.0	31

result is the mean value between those two results and represents the total CP amount in the sample, as the sum of both intermediate results would be a gross overestimation (Fig. 8).

Homologue Group Specific Analysis (GC-ECNI-Orbitrap-HRMS)

In 2016, a new quantification method using the GC-ECNI-Orbitrap-HRMS technology was established for CP at the CVUA Freiburg. The very high mass resolution of this instrument allowed for a differentiation between SCCP and MCCP while operation in full scan mode as well as reduced analysis time compared to other GC-ECNI-MS methods. The choice of the comparatively softer ionization method additionally allowed for detection of the molecular or pseudo-molecular ions and a variety of other fragment ions, offering further insight into the homologue group distribution and patterns of each sample. The specific parameters applied for CP analysis have been described in several publications (Krätschmer et al. 2018; Mézière et al. 2020; EURL for Halogenated POPs in Feed and Food 2021) and are summarized in Table 8.

While it is possible to determine homologue group specific peak areas for $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$ fragment ions with this method, currently no suitable standards with defined homologue group concentrations are available.

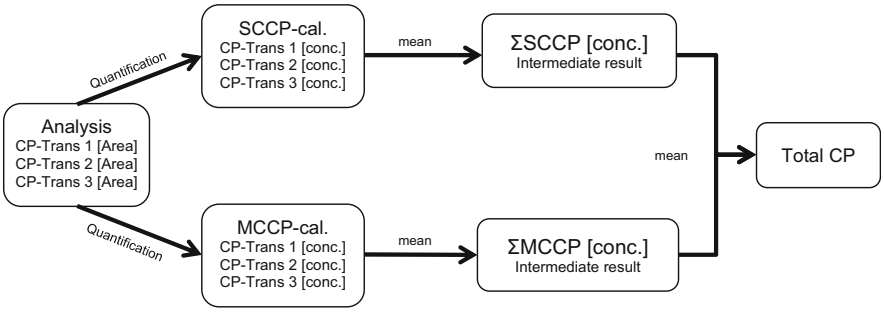

Fig. 8 Schematic quantification workflow for CP analysis using GC-EI-MS/MS

Table 8 Method parameters for CP quantification using GC-ECNI-Orbitrap-HRMS.

Method parameters: GC-ECNI-Orbitrap-HRMS (Krätschmer et al. 2018)	
Instrument	TRACE 1310 GC system coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA)
Column	15 m × 0.25 mm, 0.25 µm HP-5MS UI capillary column connected to 1 m of an uncoated pre-column (Agilent Technologies, Santa Clara, CA, USA)
GC oven programme	60 °C (2 min), increase at 50 °C/min to 300 °C (11 min) Total time 17.8 min
Detection	Electron capture negative ion (ECNI), reaction gas: methane
Data acquisition	Full scan mode (*m/z* 250–810), R = 120,000 (FWHM), extraction of three most abundant isotopologues of $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$ adduct ions for each homologue via TraceFinder software (Thermo Fisher Scientific, Waltham, MA, USA)

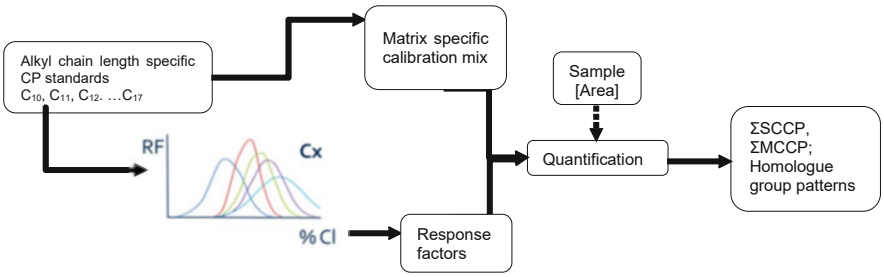

Fig. 9 Schematic quantification workflow for CP analysis using GC-ECNI-Orbitrap-HRMS

Therefore, the quantification strategy applied to the GC-ECNI-Orbitrap-HRMS data works in three interdependent steps (Fig. 9):

1. Use of alkyl chain length specific CP standards of different chlorination degrees to model homologue group specific response factors according to Yuan et al. 2017
2. Use of these response factors to characterize an SCCP and MCCP mixture of alkyl chain length specific standards designed to resemble the homologue group pattern of the target sample matrix (e.g. fish)
3. Use of the characterized standard mixture as linear or exponential calibration curve for sample quantification

This approach allows for maximum flexibility to incorporate day-to-day changes in the analytical system while allowing for first indications even of homologue group specific concentrations and reliable CP alkyl chain length specific results. For easier comparison to other studies, results reported for the human milk samples were summarized as ΣSCCP and ΣMCCP.

5.2 Quality Control

A comprehensive quality control programme was applied to prove the long-time reliability of results between 2012 and 2021.This included spiked samples at different levels and different kinds of quality control samples. Possible systematic errors were checked by analysis of reference material or participation in several interlaboratory studies. This validation is part of the general quality control programme applied in the daily routine for analysis of all kinds of samples. Analyses were performed by different operators using different chemicals over a long time and data collected in separate runs—therefore, these validation data are much more robust than that obtained from a single initial validation when one technician performs repeated analyses under the same conditions using the same chemicals in

one sequence. An overview spanning results of the quality control for the whole period 2000–2019 is summarized in the following section.

5.2.1 Initial Method Validation

Method Validation: GC-EI-MS/MS

For initial method validation, raw cow's milk was fortified with a CP standard at two different levels: 2 ng/g milk (=1 ng/μL injected sample) and 10 ng/g milk (=5 ng/μL injected sample). Additionally, chemical blanks and milk blanks were analysed with each sample batch. The fortified samples were prepared and analysed five times each. Table 9 describes the method criteria derived from this validation study.

As the sample preparation method cannot eliminate the blank levels completely, they are used for determining a limit of detection (LOD) and limit of quantification (LOQ). For calculation purposes, the blank levels of sample batches spanning at least 3 months were taken into account and LOD and LOQ values were calculated according to the following formula:

$$\mathrm{LOD} = x_{\mathrm{blank}} + 3 \times s_{\mathrm{blank}} \quad (1)$$

$$\mathrm{LOQ} = x_{\mathrm{blank}} + 10 \times s_{\mathrm{blank}} \quad (2)$$

where x_{blank} is the mean blank level [ng/μL] and s_{blank} the standard deviation [ng/μL] of the blank levels taken into account for the calculation.

During initial method validation, a mean blank level of 0.12 ng/μL with a standard deviation of 0.05 ng/μL resulted in the following parameters:

- LOD = 0.12 ng/μL + 3 × 0.05 ng/μL = 0.26 ng/μL injected sample (= 0.5 ng/g milk)
- LOQ = 0.12 ng/μL + 10 × 0.05 ng/μL = 0.62 ng/μL injected sample (= 1.2 ng/g milk)

The dilution factor used to convert ng/μL injected sample into ng/g milk includes the initial sample weight (50 g) and final sample volume before injection (100 μL).

Table 9 Validation results and resulting parameter for GC-EI-MS/MS

Parameter	Level 1 (2 ng/g)	Recovery (%)	Level 2 (10 ng/g)	Recovery (%)
Sample 1	1.82	91	9.50	95
Sample 2	1.66	83	10.0	100
Sample 3	1.46	73	9.30	93
Sample 4	2.42	121	9.74	97
Sample 5	2.06	103	11.7	117
Mean [ng/g]	**1.88**	**94**	**10.1**	**101**
SD [ng/g]	**0.37**		**0.96**	
RSD [%]	**20**		**10**	

Using similar dilution factors, the LOD and LOQ can also be applied to other matrices for a first indication of the working range.

Method Validation: GC-ECNI-Orbitrap-HRMS

For initial method validation, coconut fat and lard were fortified with SCCP and MCCP standards at different levels (Table 10).

These samples were also part of different international interlaboratory studies. For the purpose of this validation study, both samples were analysed in triplicate with accompanying chemical blank samples. Tables 11 and 12 show the results and performance with regard to recovery and RSD as important method criteria derived from this validation study.

As the sample preparation method cannot eliminate the CP background (blank level) completely, they are used for determining a limit of detection (LOD) and limit of quantification (LOQ). For calculation purposes, the blank levels of sample batches

Table 10 Fortification levels for initial method validation

Matrix	SCCP [ng/g]	MCCP [ng/g]
Lard	69	56
Coconut fat	120	180

Table 11 Results and validation parameters for the coconut fat samples

	SCCP [ng/g]	Recovery [%]	MCCP [ng/g]	Recovery [%]	sum CP [ng/g]	Recovery [%]
Sample 1	131	109	174	97	306	102
Sample 2	119	99	169	94	289	96
Sample 3	129	107	173	96	301	100
Mean [ng/g]	**126**	**105**	**172**	**96**	**299**	**100**
SD [ng/g]	**6.6**		**2.6**		**9.0**	
RSD [%]	**5**		**2**		**3**	

SD, standard deviation; RSD, relative standard deviation; sum CP, sum of detected SCCP and MCCP

Table 12 Results and validation parameters for the lard samples

	SCCP [ng/g]	Recovery [%]	MCCP [ng/g]	Recovery [%]	sum CP [ng/g]	Recovery [%]
Sample 1	69.0	100	49.3	88	118	95
Sample 2	67.5	98	44.7	79	112	90
Sample 3	73.4	107	47.1	84	121	96
Mean [ng/g]	**70.0**	**102**	**47.0**	**84**	**117**	**94**
SD [ng/g]	**3.1**		**2.3**		**4.3**	
RSD [%]	**4**		**5**		**4**	

SD, standard deviation; RSD, relative standard deviation; sum CP, sum of detected SCCP and MCCP

Table 13 Overview of limit of detection (LOD) and limit of quantification (LOQ) determined during initial method validation based on 14 blank levels analysed over the duration of 3 months

	Mean [ng/μL]	SD [ng/μL]	LOD [ng/μL]	LOD [ng/g]	LOQ [ng/μL]	LOQ [ng/g]
sum CP	0.018	0.018	0.07	4.7	0.20	13.3
SCCP	0.008	0.009	0.04	2.7	0.10	6.7
MCCP	0.009	0.014	0.05	3.3	0.15	10.0

Values in ng/g indicate LOD and LOQ calculated for human milk samples (ng/g lipid)

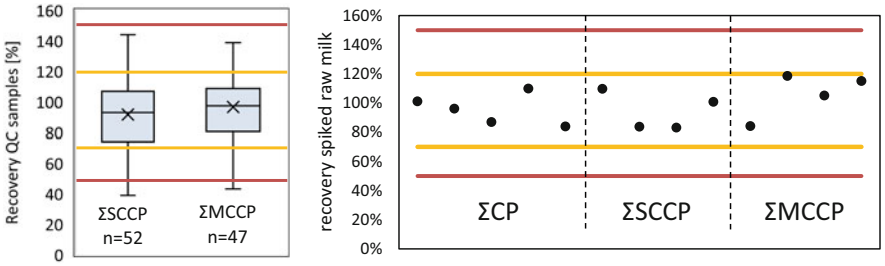

Fig. 10 Recoveries of several different QC samples analysed 2016–2020 (left) and fortified raw cow's milk samples analysed in tandem with each human milk batch 2017–2020 (right). The yellow line indicates warning levels for daily quality control, red lines equal warning limits of current CP interlaboratory studies

spanning the last 2 years on a rolling scheme were taken into account and LOD and LOQ for SCCP, MCCP and the sum of CP were calculated according to Eqs. (1) and (2). Table 13 gives an overview of the mean blank levels and resulting LODs and LOQs during the initial validation period.

5.2.2 Quality Control Samples

Between 2017 and 2020, several different matrices were prepared as quality control samples in order to provide a good fit with the sample matrix. In the case of human milk sample, raw cow's milk was analysed as the procedural blank matrix and this was fortified with SCCP and MCCP standards. Beside such matrix-specific QC samples, fortified coconut fat or lard samples from the 2017 and 2018 interlaboratory studies on SCCP and MCCP in food were routinely added to each sample batch and can therefore give a more accurate view of long-term stability and repeatability of the method (Fig. 10).

5.2.3 Participation in Interlaboratory Studies

Interlaboratory studies and proficiency tests on chlorinated paraffins in biota are to this day very rare. The performance of the GC-EI-MS/MS method was compared to other laboratories in the interlaboratory testing scheme organized by QUASIMEME 2011–2017 (van Mourik et al. 2018), which focused on SCCP in environmental matrices and standard solutions. Additionally, EURL POPs organized yearly

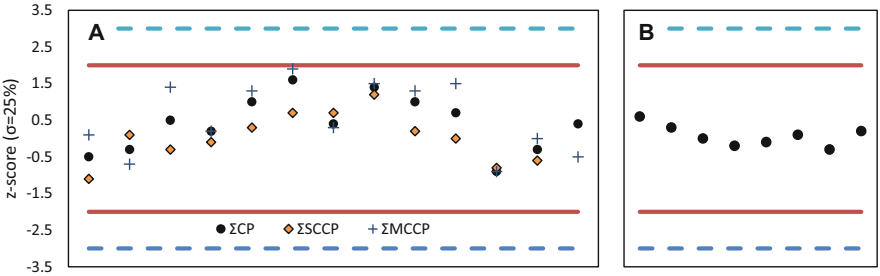

Fig. 11 Results of interlaboratory studies and proficiency tests on the determination of CP, SCCP and MCCP 2017–2020. The *z*-scores were calculated using a fitness-for-purpose-based standard deviation for proficiency tests $\sigma = 25\%$. Results within ± 2 *z* are deemed satisfactory. (**a**) Results achieved using the GC-ECNI-Orbitrap-HRMS method, (**b**) results for ΣCP achieved using the GC-EI-MS/MS method

interlaboratory studies on SCCP and MCCP in food matrices starting 2017, including CP later on in their POPs proficiency tests as optional analytes (EURL for Dioxins and PCBs in Feed and Food 2018; Krätschmer and Schächtele 2019; EURL for Halogenated POPs in Feed and Food 2019, 2020a, b).

The GC-ECNI-Orbitrap-HRMS method was used in this second study scheme as the other method was incapable of determining SCCP and MCCP separately. Due to the very complex and specialized field of analysis, the number of participants in each study was comparatively lower than for well-established analyte groups, leading sometimes to evaluations being provisional or in some cases completely impossible. Figure 11 shows all *z*-scores achieved in interlaboratory comparisons for the duration of the human milk studies discussed in the results for chlorinated paraffins. As conclusion, all *z*-scores (13 samples analysed for ΣCP, ΣSCCP and ΣMCCP using the GC-ECNI-Orbitrap-HRMS method, 8 results achieved for ΣCP using the GC-EI-MS/MS method) were within ±2 z and therefore deemed satisfactory.

6 Polychlorinated Naphthalenes (PCN)

6.1 Analytical Procedure and Analytes

For extraction of the lipids, approx. 200 g of human milk sample at approx. 4 °C was centrifuged in stainless steel centrifuge tubes for 10 min. at approx. 3000 rpm. The supernatant cream layer was transferred into a glass beaker. Sodium sulphate was added to the cream with grinding until the material was powdery when stirred. This powder was extracted 3–4 times with *n*-hexane by stirring well with a glass rod and filtering the extract. The solvent was evaporated by rotary evaporation to give the extracted lipids. The lipids were dried in a nitrogen stream on a sand bath at approx. 80 °C for 30 min and in a drying oven at 103 °C for 30 min.

A 2 g aliquot of this extract was spiked with $^{13}C_{10}$-labelled standards and dissolved in 8 mL hexane. Table 20 (in the appendix) lists the 26 determined native

congeners and the eight $^{13}C_{10}$-labelled standards that were used. Seven $^{13}C_{10}$-labelled standards listed in Table 20 in bold were added as internal standards to the lipid aliquot. $^{13}C_{10}$-labelled PCN 65 listed in Table 20 was used as the recovery standard and added after clean-up before the final determination step. The congeners were chosen based on the toxicological characteristics, reported levels of occurrence, congener patterns and the availability of analytical standards. Thus, as an example, congener 54 was not included in the target scope even if relative potency factors (REP) were reported (Fernandes et al. 2017) as the single PCN 54 standard was not available at the beginning of method development. As the availability of standards continues to improve, it is recommended and planned to extend the scope to other relevant congeners. Standards were obtained from LGC Standards (Wesel, Germany) and diluted to the appropriate levels in toluene.

Samples were purified by a fully automated clean-up system (DEXTech Plus, LCTech Obertaufkirchen, Germany) using three columns:

- Standard multi-layer silica sulphuric acid column
- Alumina column
- Carbon column

The method for determination of PCN uses the same columns and eluents as described above in the method for PBDE (Sect. 3.3.2) and for the determination of PCDD/PCDF and PCB. Pre-packed columns were provided ready-to-use by the supplier. The sample extract was loaded with hexane first onto the multi-layer silica column, then transferred with hexane onto the alumina column. PCN were eluted from the alumina column by a mixture of n-hexane and dichloromethane (1/1, v/v) onto the carbon column. The target PCN were eluted by toluene as fraction 2, spiked with the $^{13}C_{10}$-labelled recovery standard, evaporated to near dryness (~0.5 mL) and transferred into a vial. The final extract was gently blown off with nitrogen to a final volume of 50 μL. 25 μL of the final extract was transferred to a second vial and stored for a second, confirmation measurement.

The measurements were carried out using HRGC/HRMS (Trace 1310 GC coupled to DFS MS, Thermo Fisher Scientific, Waltham, MA, USA) at a resolution of 10,000 (at 5% peak height) and quantified against a 5-point calibration curve. PCN congeners (5 μL injection volume) were separated on a DB5-MS GC column (Agilent Technologies, Santa Clara, CA, USA). Confirming measurement was carried out using the same column in a GC-Orbitrap Q Exactive MS (Thermo Fisher Scientific, Waltham, MA, USA) at a resolution of 60,000 (FWHM @ m/z 200) and quantified against a 4-point calibration curve.

Five congener pairs (PCN 28/36, 52/60, 64/68, 66/67 and 71/72) could not be separated using the existing set-up, and although five other columns (Rtx-Dioxin2, Rtx-PCB, Rtx-2330 [Restek, Bellefonte, PA, USA] and DB-Dioxin, ZB-Dioxin [Phenomenex, Torrence, CA, USA]) were tested during the earlier method development phase, none of them were able to separate all the co-eluting congeners. In particular, PCN 66/67 could not be separated with any of the tested conventional chromatography columns. Columns used by other laboratories, i.e., ZB-1701 P fused

silica column (Phenomenex, Torrence, CA, USA), were similarly not suitable to separate PCN 66/67 (Zacs et al. 2021). Only a special GC column (Rt-βDEXcst, Restek, Bellefonte, PA, USA) could separate PCN 66 and 67, however, with considerable limitations under routine conditions (long GC run times of about 100 min, short column lifetime, high column bleeding and therefore increased maintenance of the MS ion source) (Helm 2002). Furthermore, this special GC column was used for separation of closely eluting PCN congeners including PCN 66 and 67 in technical mixtures by two-dimensional GC/quadrupole mass spectrometric detection (GC × GC/qMS) on Rt-βDEXcst and DB-Wax phases. However, no quantitative data were given on the composition, and neither PCN 66 nor 67 was shown in the figures on relative abundance (Hanari et al. 2013); for other stationary phases, see Fernandes et al. (2017). As a conclusion, the determination of the concentration of the individual congeners PCN 66 and 67 in human milk is not possible under routine conditions and requires research for development of a practical and valid method with sufficient sensitivity.

6.1.1 Sum Parameter for Selected PCN

Concentrations of the sum of 26 congeners (Table 20) were calculated and are part of the report on PCN in human milk (Tschiggfrei et al. 2023).

It was observed that the number of congeners reported in the literature varies for different reasons (i.e. lack of standards depending on when determinations were carried out, knowledge on occurrence and toxicology, analytical feasibility). At least the following congeners (including congeners in co-eluting pairs) were reported frequently in literature (Fernandes et al. 2017): PCN 52/60, 53, 66/67, 64/68, 69, 71/72, 73, 74 and 75. These 13 congeners show toxicological relevance due to high REP factors up to 0.004 for PCN66/67 (Falandysz et al. 2019; Fernandes et al. 2010, 2011, 2017, 2022b, d; Zhihua et al. 2019; Zacs et al. 2021). In order to give an additional overview of the quality control with focus on these congeners, the sum of these 13 congeners was calculated in addition to the sum of 26 congeners and is used for certain quality control parameters.

6.1.2 Toxic Equivalents (TEQ)

Toxic Equivalents (TEQ) were calculated as the sum of the products of the concentration of each compound (26 congeners) multiplied by the corresponding aryl hydrocarbon receptor-mediated (dioxin-like) relative potency factors (REP) and provided an estimate of the 2,3,7,8-TCDD-like activity.

REP values for PCN suggested by Falandysz et al. 2014 and REPs used in human exposure studies (Falandysz et al. 2019; Falandysz and Fernandes 2020; Fernandes et al. 2010, 2011, 2017, 2022b, d; Pratt et al. 2013; Zhihua et al. 2019; Zacs et al. 2021) were used for the estimation of PCN-TEQ. The applied REPs are compiled in the article on PCN in human milk (Tschiggfrei et al. 2023).

6.1.3 Limits of Quantification and Acceptable Differences Between Upper-Bound and Lower-Bound Results for PCN-TEQ

The limit of detection (LOD) and/or limit of quantification (LOQ) are important parameters for the evaluation of the reliability of analytical results. Due to similar analytical attributes between polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and PCN, the requirements for the determination of the LOQ for PCDD/PCDF in food according to European legislation were taken as guidance. Thus, the following conditions were followed to determine the LOQ for all 26 PCN congeners (European Commission 2012):

> The accepted specific LOQ of an individual congener is the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions, to be monitored with an S/N (signal/noise) ratio of 3:1 for the less sensitive signal and fulfilment of the basic requirements such as retention time, isotope ratio according to the determination procedures as described in EPA method 1613 revision B.

Following the concept for reporting of TEQ results as established for PCDD/PCDF and dioxin-like polychlorinated biphenyls (dl-PCB), the upper-bound (ND=LOQ) and the lower-bound (ND = 0) values should be given (Malisch et al. 2023b; UNEP 2019). The following two harmonized quality criteria were applied:

(i) Calculation of the contribution of each non-detected congener to the TEQ as zero (lower-bound concentrations)
(ii) Calculation of the contribution of each non-detected congener to the TEQ as the limit of detection (upper-bound concentrations)

The upper- and lower-bound values have important implications for the interpretation of the analytical results (Malisch and Schächtele 2023). As a performance criterion, the difference between these two should be less than 20% (UNEP 2019). In cases of co-eluting congeners and different REPs used in human biomonitoring or as suggested by Falandysz et al. 2014, both REP factors were applied and conclusions on the differences of the PCN-TEQ results drawn. The median of the differences between lower- and upper-bound PCN-TEQ in all 40 human milk samples of the 2016–2019 period was 0.3%. The range was between 0 and 2%, if REPs as used for human biomonitoring were applied, and between 0 and 3%, if other suggested REPs were used. Therefore, all samples analysed in this study fulfilled this QA/QC criterion. These differences were considered negligible (Tschiggfrei et al. 2023).

6.2 Quality Control

Before analysing the WHO-human milk study samples on PCN, the developed method was checked for precision and trueness by a small validation study in raw milk. Possible systematic errors were checked by applying a quality control programme including procedural blank samples, two different kinds of in-house

reference material (spiked milk fat and butter) and confirmation of certain results by a different detection technique (GC-Orbitrap measurement). Quality control samples were included in the routine analysis for a broader picture of the PCN spectrum and to check the accuracy of the method. As the analysis of PCN is not widespread yet and no proficiency tests were available at the time of performance of the analyses, external validation was not possible at that time. As a substitute, contaminated fat samples with incurred PCN and a vegetable oil sample spiked with PCN were analysed by an independent laboratory to check the trueness. At a later stage, CVUA Freiburg took part in a first interlaboratory comparison study in cod liver oil for 26 PCN congeners conducted by the EURL POPs in the second half of 2021; the results are included in the following (see Sect. 6.2.5). All these steps illustrate the extent of validation, if methods have to be developed for new POPs.

6.2.1 Procedural Blank Samples

For PCN, the median of 22 procedural blank samples analysed between 2020 and 2021 is 0.61 pg/g fat (lower-bound [lb]) for ΣPCN_{26} (Table 14). In most cases, congeners were below the LOQ. Therefore, the lower-bound value of the procedural blank is a better indication of the worst-case contamination than a reagent blank which could be considered for possible subtraction. The influence of procedural blank samples was negligible for human milk samples.

6.2.2 Fortified Raw Milk and Butter Samples as In-House Reference Material

Fortification of fat or oil (i.e. sunflower oil) samples (which were tested to be free of PCN residues) with native PCN congeners at relevant levels is a well-established procedure to check the recovery of native analytes. The extracted fat of four raw milk samples was spiked at 15 pg/g lipid for each of the 26 PCN congeners. Evaluation of the recovery and precision was carried out by calculating the coefficient of variation (CV), before starting the analyses of the WHO/UNEP-human milk samples. Additionally, seven butter samples spiked at 15 pg/g lipid for each of the 26 PCN congeners were evaluated together with the performance of the analyses of the human milk samples. The mean recoveries for milk fat and butter for ΣPCN_{13} and ΣPCN_{26} were in the range 100–101% with a CV of the recovery in the range 1–2%

Table 14 ΣPCN_{26} (pg/g lipid) levels and ΣPCN_{13} (pg/g lipid) levels of reagent blank samples analysed together with human milk and fatty food samples between 2020 and 2021

	ΣPCN_{26} (lb) pg/g fat	ΣPCN_{13} (lb) pg/g fat
Number of analyses	22	22
Median	0.61	0.13
Mean	1.02	0.20
25%-percentile	0.38	0.08
75%-percentile	1.18	0.26
90%-percentile	2.31	0.33
95%-percentile	3.71	0.64
Maximum	4.25	0.84

Table 15 Recovery and CV (%) of samples fortified at 195 pg/g lipid ΣPCN_{13} and 390 pg/g lipid ΣPCN_{26} respectively, analysed before (milk fat) and together (butter) with human milk samples

Fortified level pg/g lipid		Matrix	No of replicates	Mean	Recovery (%)	CV (%)
ΣPCN_{13}	195	Milk fat	4	197	101	1
ΣPCN_{26}	390	Milk fat	4	395	101	0.9
ΣPCN_{13}	195	Butter	7	196	100	2
ΣPCN_{26}	390	Butter	7	390	100	2

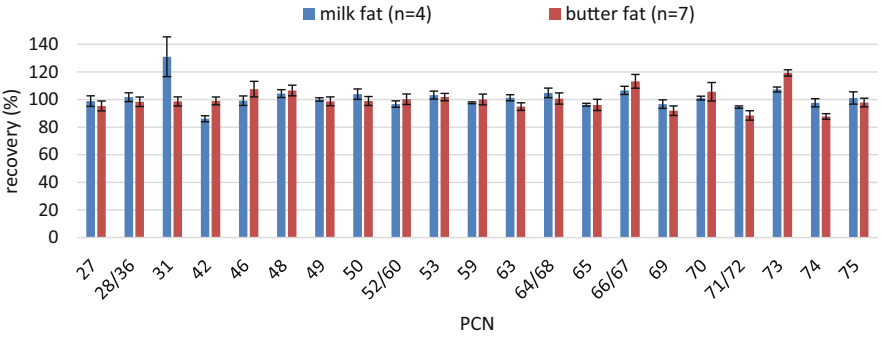

Fig. 12 Recovery and CV (%) of samples fortified at 15 pg/g lipid for each of the 26 PCN congeners analysed before (milk fat) and together (butter) with human milk samples

(Table 15). For each of the congeners, the recoveries were between 86% (PCN 42) and 131% (PCN 31) with a CV between 0.7 and 11% (Fig. 12).

6.2.3 Quality Control Samples as In-House Reference Material and Precision

In order to control the analytical performance at different concentration levels of PCN congeners, a quality control sample (fish oil contaminated at different levels of single PCN congeners) was analysed together with human milk samples. In this way, the linearity of the response for PCN (range between 0.2 pg/g lipid for PCN 70 and 130 pg/g lipid for PCN 52/60) was checked. The fish oil, with a concentration of 324 pg ΣPCN_{26}/g lipid, had on average 2–3 times higher concentrations of ΣPCN_{26} than human milk (26–170 pg ΣPCN_{26}/g lipid; ΣPCN_{13} 20–134 pg/g lipid; Tschiggfrei et al. 2023), but covers the same order of magnitude as findings reported in the literature based on the determined congeners (483–3081 pg ΣTetraCN-OctaCN/g lipid (Lundén and Norén 1998), 59–168 pg ΣPCN_{12}/g lipid (Pratt et al. 2013), 211–2497 pg ΣMonoCN-OctaCN/g lipid (Li et al. 2020).

Over the whole period of its use from 2019 until 2021, 24 replicates of the fish oil were analysed under intermediate precision conditions, i.e., by different technicians under varying conditions, various batches of chemicals, instrumental conditions, etc. Table 16 presents the coefficient of variation (CV) obtained by repeated analysis of

Table 16 CV (%) of the quality control sample "fish oil" at 324 pg ΣPCN_{26}/g lipid and 220 pg ΣPCN_{13}/g lipid, respectively, used between 2019 and 2021

Fish oil sample	Mean level (pg/g lipid)	No. of replicates	CV (%)
ΣPCN_{26}	324	24	7
ΣPCN_{13}	220	24	6

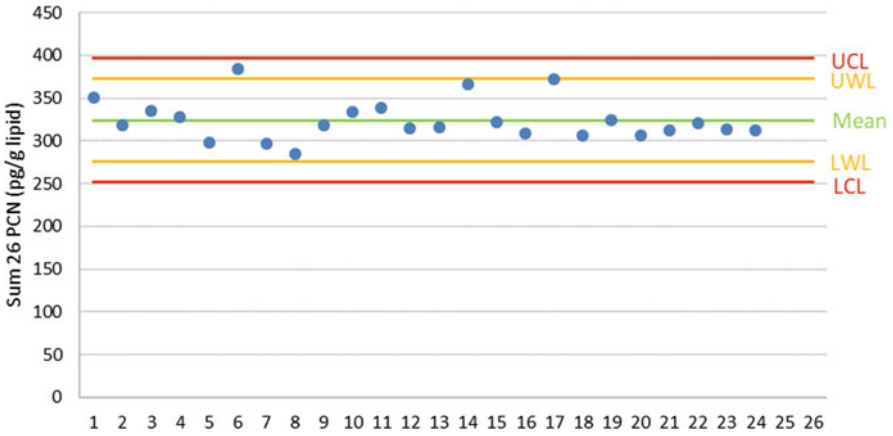

Fig. 13 Quality control chart for fish oil (324 pg ΣPCN_{26}/g lipid) used from 2019 to 2021 (UCL, upper control level; UWL, upper warning level; LWL, lower warning level; LCL, lower control level)

this quality control sample, indicating a high precision of the analytical method. In the 3 years of use of this quality control sample, a CV of 7% was observed for the mean level for ΣPCN_{26} of 324 pg/g lipid, a CV of 6% for the mean level for ΣPCN_{13} of 220 pg/g lipid.

Figure 13 illustrates the quality control charts for ΣPCN_{26} resulting from use of this fish oil over the whole period of its use from 2019 until 2021. Around the mean (M), warning levels are set at two sigma (lower warning level at M-2s, upper warning level at M+2s), control levels at three sigma (lower control level at M-3s, upper control level at M+3s). The upper warning level of 372 pg ΣPCN_{26}/g lipid was reached by one of the 24 replicates, the lower warning level of 276 pg ΣPCN_{26}/g lipid was not exceeded. None of the 24 replicates exceeded the lower (252 pg ΣPCN_{26}/g lipid) or upper (387 pg ΣPCN_{26}/g lipid) control level.

In addition to these conclusions from the general quality control of the PCN analyses performed between 2019 and 2021, it should be noted that the quality control fish oil samples that were analysed together with the human milk samples did not show exceedance of any warning level or control level of the individual 26 PCN congeners.

As a result, based on this quality control sample, the applied method achieved a long-term precision of below 10% over the 2019–2021 period for ΣPCN_{13} and ΣPCN_{26}.

6.2.4 External Validation

Due to the lack of available proficiency tests at the time of measuring the human milk samples of the 2016–2019 period, an external validation for control of the trueness was performed through an interlaboratory comparison with an independent laboratory. Results of four highly polluted fat samples with incurred PCN and a fortified rape seed oil (ΣPCN_{13} 195 pg/g lipid) were compared. At CVUA Freiburg, PCN were determined by GC-HRMS (high-resolution mass spectrometry; sector field instrument, R = 10,000) and confirmed by GC-Orbitrap MS (Orbitrap mass spectrometry; R = 60,000) (see Sect. 6.1). The external laboratory also used a sector field mass spectrometer (R = 10,000) for PCN determination. Both laboratories analysed at least 13 of the most frequently reported congeners (see Sect. 6.1). The deviation for the results between the external laboratory and CVUA Freiburg is summarized in Table 17. The deviation of the ΣPCN_{13} between the external laboratory and CVUA Freiburg was in the range between 3 and 20%.

The PCN pattern achieved by both labs were in agreement (Fig. 14). An overestimation using GC-Orbitrap for PCN 28/36 and PCN 48 in comparison with sector field detection was recognized and may have been caused by a possible co-elution of interfering compounds or other PCN congeners.

6.2.5 Participation in Interlaboratory Studies

At a later stage, CVUA Freiburg took part in the first interlaboratory comparison study conducted by the EURL POPs in the second half of 2021 on determination of PCN in cod liver oil for 26 PCN congeners (EURL for Halogenated POPs in Feed and Food 2022b). The number of participants in this first interlaboratory study for PCN was lower than for well-established analyte groups. Furthermore, the scope on targeted PCN congeners varied between the participating labs, limiting the possibility of assessment of the comparability for some congeners. Assigned values could be derived for 7 congeners/co-eluting pairs of congeners and two sum parameters

Table 17 Deviation (%) of the results of ΣPCN_{13} between CVUA Freiburg and an external laboratory both using high-resolution mass spectrometry (resolution 10,000) for determination of PCN in four contaminated samples (two of them highly) and one fortified sample

Sample	Mean level between the two labs ΣPCN_{13} (pg/g lipid)	No of replicates CVUA Freiburg/ external lab	Deviation between the two labs (%)
Human milk	1000	4/4	19
Falcon egg	7847	2/3	15
Cod liver oil	550	2/5	20
Fish oil	220	7/2	3
Fortified rapeseed oil	193	2/6	11

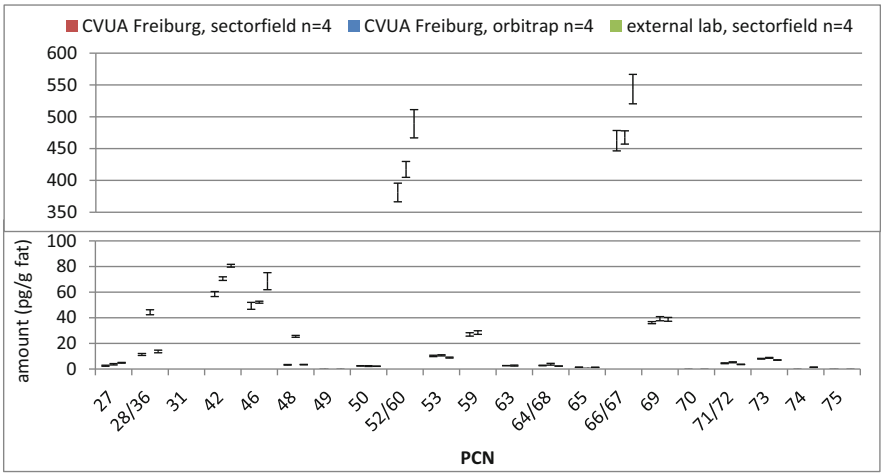

Fig. 14 Comparison of a PCN pattern of a highly polluted human milk sample by CVUA Freiburg using GC-Orbitrap (R = 60,000) and sector field (R = 10,000) and an external lab using sector field (R = 10,000). The congeners PCN 31, PCN 59 and PCN 63 were only determined by CVUA Freiburg

([1] "ΣPCN_{12} plus PCN 64" comprising the 12 main congeners recommended by the PT provider as initial focus when starting the method development plus PCN 64; [2] ΣPCN_{26} comprising all 26 congeners) (Table 18). Figure 15 illustrates the *z*-scores achieved in this interlaboratory comparison using the sector field method. As a conclusion, all *z*-scores were within ±2 *z* and therefore deemed satisfactory.

Additionally, the deviation of the results to the median was calculated as useful information in particular for congeners/co-eluting pairs of congeners, if no assigned value and thus no *z*-score could be calculated. This information is seen as meaningful, if results from five or more laboratories for concentrations above the lowest background level (>0.3 pg/g lipid) were available. In general, the results for 26 PCN congeners from CVUA Freiburg were in accordance with the median of the results of the participants (Table 18).

7 Accreditation

In 1993, new quality standards were introduced for laboratories entrusted with the official control of foodstuffs by the Member States of the European Economic Community. Laboratories had to comply with the general criteria for the operation of testing laboratories laid down in European Standard EN 45001 supplemented by standard operating procedures and the random audit of their compliance by quality assurance personnel not later than November 1998 (Council Directive 93/99/EEC). In a revision of the regulations on official controls in 2004, it was stipulated that laboratories that were designated for official control should operate and be assessed

Table 18 Results of the interlaboratory study on the determination of PCN in cod liver oil

Parameter	Assigned value (AV)[a] pg/g lipid	No. of results for AV	Median [all values] pg/g lipid	No. of results for median	Achieved *z*-score	Deviation of results to median[b]
PCN 27			1.18	9		0%
PCN 28/36			3.40	7		0 %
PCN 31			0.450	3		(–)[c]
PCN 42	28.2	9	28.1	9	0.4	
PCN 46			1.80	6		3%
PCN 48			0.451	6		–5%
PCN 49			0.325	6		21%
PCN 50			1.76	6		–9%
PCN 52*/60*	49.7	9	48.3	10	0.5	
PCN 53*			2.94	10		19%
PCN 59			4.10	3		(–)[c]
PCN 63			0.630	4		(–)[c]
PCN 64/68*	2.22	8	2.41	10	1.8	
PCN 65			0.493	7		–5%
PCN 66*/67*	7.54	7	7.46	10	0.7	
PCN 69*	2.38	8	2.39	9	0.6	
PCN 70			0.200	7		(–)[c]
PCN 71*/72*			2.14	10		26%
PCN 73*	1.24	7	1.33	10	0.8	
PCN 74*			0.418	8		–8%
PCN 75*	0.581	9	0.571	10	1.3	
ΣPCN_{26} (ub)	103	8	99.9	10	0.9	
ΣPCN_{26} (lb)	102	8	98.2	10	1.0	
ΣPCN_{12}[d] (ub)	67.7	8	67.8	10	0.8	
ΣPCN_{12}[d] (lb)	67.2	8	66.9	10	0.8	

The *-marked PCN congeners were recommended by the PT provider as an initial focus when starting method development. Assigned values (AV) and *z*-scores could be calculated for 7 congeners/co-eluting pairs of congeners and two sum parameters. Median values were calculated for 26 congeners/congener pairs. The calculation of the deviation of the results from the median values is meaningful in cases of sufficient number of results (minimum 5 samples) and above concentrations of 0.3 pg/g lipid

[a]Huber robust mean (outliers removed)

[b]The median value was taken for comparison of the results whenever no assigned value could be calculated (possible reasons: high variation of participants' results; less than 2/3 of all results were above the LOQ; more than 1/3 of all results (including LOQs) were outside the range of ± 50% of the median of all reported results). Only median values calculated from >4 results and concentrations above 0.3 pg/g lipid were taken for comparison

[c](–) = not meaningful, as not meeting criteria for calculation of the deviation of results to median

[d]Sum of *12 main congeners plus PCN 64

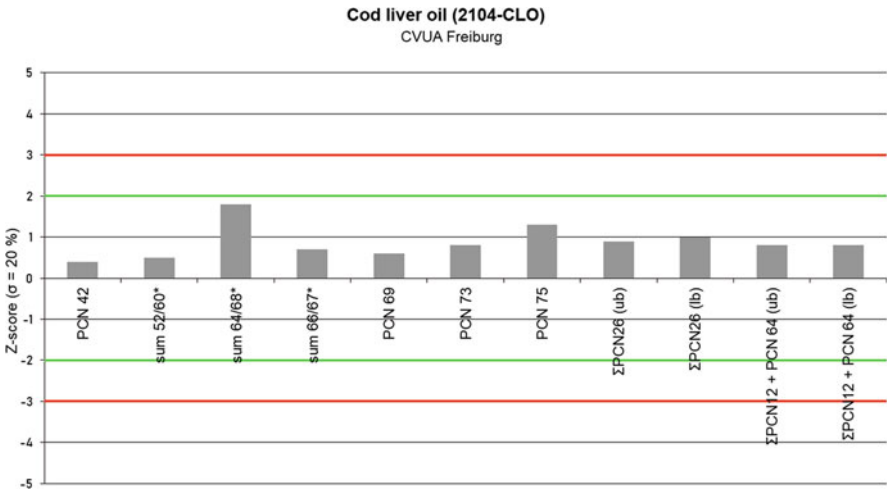

Fig. 15 Results of the interlaboratory study on the determination of PCN in cod liver oil. The plot shows *z*-scores, calculated for a fitness-for-purpose-based standard deviation for proficiency tests $\sigma = 20\%$. Results within ± 2 *z*-score are deemed satisfactory

and accredited in accordance with the European Standard EN ISO/IEC 17025—"-General requirements for the competence of testing and calibration laboratories" (EU Regulation 882/2004). Therefore, the CVUA Freiburg was accredited in 1998 and has since been re-accredited continuously.

As a result, all analyses performed by CVUA Freiburg for determination of PBDE, HBCDD, CP and PCN in human milk of the WHO/UNEP-coordinated exposure studies followed the strict rules of the accreditation system and the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025 (European Standard EN ISO/IEC 17025).

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratory, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory and administrative staff.

We thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects during her time at the United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

We greatly appreciate the performance of the analyses by the team of the residue department at CVUA Freiburg. Alwyn Fernandes, University of East Anglia, UK, is gratefully acknowledged for his editorial support.

Appendix

Table 19 25 Native and 13 $^{13}C_{12}$-labelled PBDE. Recommended analytes (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209) are marked in bold

		Native	$^{13}C_{12}$-labelled
BDE 15	4,4′-DiBDE	x	x
BDE 17	2,2′,4-TriBDE	x	
BDE 28	2,4,4′-TriBDE	x	
BDE 47	**2,2′,4,4′-TetraBDE**	**x**	**x**
BDE 49	2,2′,4,5′-TetraBDE	x	
BDE 66	2,3′,4,4′-TetraBDE	x	
BDE 75	2,4,4′,6-TetraBDE	x	
BDE 77	3,3′,4,4′-TetraBDE	x	x
BDE 85	2,2′,3,4,4′-PentaBDE	x	
BDE 99	**2,2′,4,4′,5-PentaBDE**	**x**	**x**
BDE 100	**2,2′,4,4′,6-PentaBDE**	**x**	**x**
BDE 119	2,3′,4,4′,6-PentaBDE	x	
BDE 126	3,3′,4,4′,5-PentaBDE	x	x
BDE 138	2,2′,3,4,4′,5′-HexaBDE	x	
BDE 153	**2,2′,4,4′,5,5′-HexaBDE**	**x**	**x**
BDE 154	**2,2′,4,4′,5,6′-HexaBDE**	**x**	**x**
BDE 183	**2,2′,3,4,4′,5′,6-HeptaBDE**	**x**	**x**
BDE 190	2,3,3′,4,4′,5,6-HeptaBDE	x	
BDE 196	2,2′,3,3′,4,4′,5,6′-OctaBDE	x	
BDE 197	2,2′,3,3′,4,4′,6,6′-Octa BDE	x	x
BDE 203	2,2′,3,4,4′,5,5′,6-OctaBDE	x	
BDE 206	2,2′,3,3′,4,4′,5,5′,6-NonaBDE	x	x
BDE 207	2,2′,3,3′,4,4′,5,6,6′-NonaBDE	x	x
BDE 208	2,2′,3,3′,4,5,5′,6,6′-NonaBDE	x	
BDE 209	**2,2′,3,3′,4,4′,5,5′,6,6′-DecaBDE**	**x**	**x**

Table 20 26 native and eight $^{13}C_{10}$-labelled PCNs. Seven $^{13}C_{10}$-labelled standards marked in bold were added as internal standards to the lipid aliquot; $^{13}C_{10}$-labelled PCN 65 was used as the recovery standard and added after clean-up before the final determination step

		TEF	Native	$^{13}C_{10}$-labelled
PCN 27	**1,2,3,4-TETRACN**		X	**X**
PCN 28	1,2,3,5-TETRACN		X	
PCN 31	1,2,3,8-TETRACN		X	
PCN 36	1,2,5,6-TETRACN		X	
PCN 42	**1,3,5,7-TETRACN**		X	**X**
PCN 46	1,4,5,8-TETRACN		X	
PCN 48	2,3,6,7-TETRACN		X	
PCN 49	1,2,3,4,5-PENTACN		X	
PCN 50	1,2,3,4,6-PENTACN		X	
PCN 52	**1,2,3,5,7-PENTACN**		X	**X**
PCN 53	1,2,3,5,8-PENTACN		X	
PCN 59	1,2,4,5,8-PENTACN		X	
PCN 60	1,2,4,6,7-PENTACN		X	
PCN 63	1,2,3,4,5,6-HEXACN		X	
PCN 64	**1,2,3,4,5,7-HEXACN**		X	**X**
PCN 65	1,2,3,4,5,8-HEXACN		X	X
PCN 66	1,2,3,4,6,7-HEXACN		X	
PCN 67	**1,2,3,5,6,7-HEXACN**		X	**X**
PCN 68	1,2,3,5,6,8-HEXACN		X	
PCN 69	1,2,3,5,7,8-HEXACN		X	
PCN 70	1,2,3,6,7,8-HEXACN		X	
PCN 71	1,2,4,5,6,8-HEXACN		X	
PCN 72	1,2,4,5,7,8-HEXACN		X	
PCN 73	**1,2,3,4,5,6,7-HEPTACN**		X	**X**
PCN 74	1,2,3,4,5,6,8-HEPTACN		X	
PCN 75	**OCTACN**		X	**X**

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Part III

WHO/UNEP-Coordinated Exposure Studies 2000–2019: Results of Chlorinated and Brominated POPs and Discussion

WHO- and UNEP-Coordinated Exposure Studies 2000–2019: Findings of Polychlorinated Biphenyls, Polychlorinated Dibenzo-*p*-Dioxins, and Polychlorinated Dibenzofurans

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Abstract

The concentrations of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) were determined in 232 pooled human milk samples from 82 countries from all United Nations regions participating in five exposure studies coordinated by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) between 2000 and 2019.

The highest concentrations of **PCB** were found in European countries. Countries of all other regions had considerably lower concentrations.

The highest median concentrations of **toxic equivalents (TEQ) of PCDD/PCDF and dioxin-like PCB** (expressed as **WHO_{2005}-TEQ**) were found in Eastern and Western European countries, the widest variation in Africa. The median concentrations and maximum levels in the Pacific region and countries from Latin America and the Caribbean were at the lower end of the distribution.

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_7

However, also time trends have to be considered for this overall picture for a period of 20 years.

Keywords

Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · PCB · PCDD/PCDF ("dioxins") · Global WHO/UNEP studies · UN regions · Time trends

1 Introduction

Polychlorinated biphenyls (PCB) are industrial chemicals that were manufactured for decades before their production and use was banned by many countries around 1985. Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are unintentional by-products formed in (1) a number of chemical processes and, therefore, found as contaminants in certain chemicals; (2) many combustion processes; and, (3) certain geological processes and are, therefore, present in certain clays. PCB and PCDD/PCDF are especially chemically and physiologically stable, and thus persist in the environmental and biological systems. They are also highly lipophilic, which results in their biomagnification in the food chain and bioaccumulation in fatty tissues of animals and humans. Therefore, PCB and PCDD/PCDF are classified as persistent organic pollutants (POPs) and were included in the initial 12 POPs listed by Stockholm Convention on Persistent Organic Pollutants (UNEP 2001). The objective of this Convention is to protect human health and the environment from POPs by reducing or eliminating releases to the environment.

In the late 1980s and early 1990s, the World Health Organization (WHO) initiated two global human exposure studies for certain congeners of PCB and PCDD/PCDF in human milk. The third round of the WHO-coordinated exposure studies was performed from 2000 to 2003. After a sufficient number of countries ratified the Stockholm Convention in 2004, the WHO and the United Nations Environment Programme (UNEP) agreed to collaborate in joint studies to support the implementation of the convention. In particular, the Article 16 requires the periodic evaluation of the effectiveness of the convention in reducing emissions of POPs, which includes the three groups of POPs in this paper. One of the pillars of this evaluation is to be based on comparable and consistent monitoring data on the presence of POPs in the environment and in humans (UNEP 2007, 2019). Therefore, four more rounds were organized by WHO and UNEP between 2005 and 2019. Note that the number of POPs initially covered by the convention was expanded considerably since its adoption (UNEP 2020).

Results of certain studies or periods were presented in individual publications, e.g. on the 2000–2003 round (Malisch and van Leeuwen 2003), the 2000–2008 period (Malisch et al. 2008), and the 2008–2009 round (Malisch et al. 2010). The worldwide presence of POPs in air and in humans was demonstrated by UNEP

projects in 32 developing countries with human milk data of the period 2008–2010 (Fiedler et al. 2013). A comprehensive report for the 6th Conference of the Parties to the Stockholm Convention on POPs in 2013 provided an overview on all samples of the third, fourth, and fifth round, spanning the period 2000–2012. It revealed large global differences among various POPs and a decreasing trend in PCDD and PCDF levels in a number of countries (UNEP 2013a). Also, aspects of a risk–benefit assessment of breastfeeding were addressed that were published later in more detail (Van den Berg et al. 2017). It indicated that human milk levels of PCDD, PCDF, and PCB were still significantly above those considered toxicologically safe, in some countries an order of magnitude. These observations provided a strong argument for a plea to further global source-directed measures to reduce human exposure to dioxin-like compounds.

All substance-specific data are contained at the POPs Global Monitoring Plan Data Warehouse (GMP DWH) and can be publicly retrieved. This serves as the source of information for the regional and global reports of the GMP and effectiveness evaluation (Global Monitoring Plan Data Warehouse 2020).

In this compendium, human milk surveys are reviewed. In five parts, specific papers address various aspects. Part I gives a review of human milk surveys on POPs (Fürst 2023), an overview of the WHO/UNEP-coordinated exposure studies performed between 1987 and 2019 (Malisch et al. 2023a), and a review on the Stockholm Convention and its implementation by regional and global monitoring reports (Šebková 2023). Part II presents the analytical aspects of these studies, including methods for PCB and PCDD/PCDF and their validation (Malisch and Schächtele 2023). In Part III, the findings between 2000 and 2019 are presented in various publications, in this paper in relation to PCB and PCDD/PCDF. Countries are assigned to one of the five United Nations (UN) regions (see Sect. 2.1). It should, therefore, be noted that these results are not intended to be used for the ranking of countries. Part IV presents assessments of time trends derived from countries with repeated participation in the WHO- and UNEP-coordinated studies, among them for PCDD/PCDF and PCB (Malisch et al. 2023b) and a review of possible health risks for the breastfed infant from dioxin-like compounds (Van den Berg et al. 2023). Part V presents conclusions and key messages.

In addition to the above-mentioned compilation (Fürst 2023), a review of scientific publications between 1995 and 2011 on the spatial and temporal trends of Stockholm Convention on POPs in breast milk can be used to compare results of PCB and PCDD/PCDF levels (Fång et al. 2015). Furthermore, the regional and global monitoring reports for the Global Monitoring Plan assess datasets in the core media—ambient air, human tissues (human breast milk or blood), and water for hydrophilic POPs, but also other media such as soil, biota, plants are used to support interpretation of observed levels and their trends (Šebková 2023). These reports are available at the homepage of the Stockholm Convention (>Implementation>Global Monitoring Plan>Monitoring Reports).

This article compiles the results of a total of 82 countries participating in one or more of the five WHO/UNEP-coordinated exposure studies conducted between 2000 and 2019 with submission of a total of 232 pooled human milk samples. As

relevant congeners, 17 PCDD/PCDF, 12 dioxin-like PCB, and 6 Indicator PCB were determined. Toxic Equivalent (TEQ) concentrations were calculated based on Toxic Equivalency Factors recommended by WHO in 2005 (see Sect. 2.4). Four main summarizing parameters are given for: (1) PCDD and PCDF as WHO-PCDD/PCDF-TEQ; (2) dioxin-like PCB as WHO-PCB-TEQ; (3) the sum of PCDD, PCDF, and dioxin-like PCB ("total TEQ") as WHO_{2005}-TEQ; and (4) the sum of 6 Indicator PCB (ΣPCB_6) as the total concentration of the 6 selected non-dioxin-like PCB (see Sect. 2.6). Note that all concentrations in this paper are expressed on a lipid basis.

These TEQ and ΣPCB_6 results give a complex but comprehensive picture of the global exposure to these POPs over the past 20 years. The results for all samples and parameters are given and discussed from various perspectives in the following sections, namely: General aspects (Sect. 2); overall comparison of concentrations of TEQ and non-dioxin-like PCB among UN regions (Sect. 3); the five WHO- and UNEP-coordinated studies from 2000 to 2019 (Sect. 4); detailed comparison of concentrations on a Regional Group scale (Sect. 5), correlation between indicator PCB and dioxin-like PCB and between dioxin-like PCB and PCDF (Sect. 6) and summary (Sect. 7).

2 General Aspects

2.1 Link to the General Introduction (Countries, UN Regions, Protocol)

An overview of the scope, protocols for collection of samples and participation of countries with classification in UN regions and temporal differentiation is given in the general introduction (Malisch et al. 2023a). Shortly, in all rounds the design was based on collection of a number of individual samples and preparation of pooled samples following a standardized protocol that was supervised by national coordinators. Equal aliquots of individual samples were combined to give composite samples, which are considered representative of the average levels of the analytes of interest in human milk for a certain country or subgroup/region of a country at the time of sampling. The pooled samples were sent to WHO/UNEP Reference Laboratories for analysis.

In accordance with the implementation of the Global Monitoring Plan (GMP), parties report flexibly through one of the five United Nations Regional Groups. Therefore, countries are classified according to one of these five UN geopolitical groups (United Nations 2019): the African Group, the Asia-Pacific Group, the Group of Latin American and Caribbean Countries (GRULAC), the Eastern European Group, and the Western European and Others Group (WEOG). Note that Australia, Israel, New Zealand, and USA (being informally a member) are included as "Others" in the WEOG category, whereas Cyprus belongs to the Asia-Pacific Group.

2.2 Number of Samples, Aggregation of Data and Analysis

During the five studies conducted from 2000 to 2019, a total of 232 pooled samples were submitted for analysis by 82 countries and analysed for PCB and PCDD/PCDF at CVUA Freiburg, Germany. The detailed data for all 232 pooled samples is contained at the POPs Global Monitoring Plan Data Warehouse and can be publicly retrieved (Global Monitoring Plan Data Warehouse 2020).

In the 2000–2003 study, countries were particularly encouraged to submit at least two pooled samples, whereas in the following rounds in most cases one pooled sample was submitted by a country. To allow a quick and easy comparison, if a country had sent two or more samples in a certain round, the median has been used for aggregation. 113 results were from a single pooled sample submitted by countries in a certain round, whereas 31 results from the following countries were aggregated from two or more samples using the median:

- Australia, 2002 and 2013
- Belgium, 2002
- Brazil, 2001 and 2012
- Bulgaria, 2001
- Croatia, 2001
- Czech Republic, 2001
- Egypt, 2001
- Fiji, 2002 and 2006
- Finland, 2001 and 2007
- Germany, 2002 and 2019
- Hong Kong, 2002 and 2009
- Hungary, 2001
- Ireland, 2001
- Italy, 2001
- Luxembourg, 2002
- Netherlands, 2001
- New Zealand, 2000
- Norway, 2001
- Philippines, 2002
- Romania, 2001
- Russia, 2001
- Slovak Republic, 2001
- Spain, 2001
- Ukraine, 2001
- USA, 2003

With the approach of "one country – one result" for a certain round, altogether 144 country results are available for 82 countries. In this article, both the country results (from 113 single pooled samples and from aggregation of data as median in the above listed 31 cases) and the ranges found without aggregation are given.

The analytical methods for determination of PCB and PCDD/PCDF and their validation are presented in Part II (Malisch and Schächtele 2023).

2.3 Cost-Effectiveness and Possible Range of Individual Samples Using Pooled Samples

An important advantage of the WHO- and UNEP-coordinated exposure studies is the cost-effectiveness of the concept (Malisch et al. 2023a). Shortly, the analysis of the 232 pooled samples obtained from between 10 and 50 individual samples and considered to be representative for countries or subgroups/regions provides the same information as would be received by calculation of the mean of more than 2000 individual samples (assuming 10 individual samples per pool) or more than 11,000 individual samples (assuming 50 individual samples per pool). Thus, the analysis of pooled samples is an extremely cost-efficient way to get information on the average levels of the relevant POPs in humans in these countries at specific times. It also saves considerable time with respect to chemical analysis and is environmentally friendly, because less extraction solvents could be used.

On the other hand, the analysis of individual samples (from specific donors) can provide information on exposure distribution in a population and on factors possibly contributing to exposure. A follow-up might be of interest in case of considerably elevated levels. As an example of the range of concentrations in individual samples, the frequency distribution derived from 271 individual human milk samples collected in Germany during the period 1995–1998 can be used as illustration of this distribution: It shows a log-normal distribution with a maximum of the curve around 16 ng I-TEQ/kg lipids (based on International Toxic Equivalency Factors) and a range of roughly between 4 and 40 ng I-TEQ/kg lipids (Fig. 1) (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit 2002).

2.4 Toxic Equivalency Factors (TEF) and Toxic Equivalents (TEQ)

Of the theoretically possible congeners of PCDD and PCDF (75 PCDD and 135 PCDF, respectively), only the 17 congeners with at least four chlorine atoms with substitution in the 2,3,7,8-positions were considered to be relevant for human health. Similarly, from the 209 theoretically possible PCB congeners, only 12 congeners (8 mono-ortho substituted and 4 non-ortho substituted) have dioxin-like properties. These congeners show different toxic potencies that are expressed as toxic equivalency factors (TEF) compared to the most toxic congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). With the TEF, the toxicity of a mixture of PCDD/PCDF and dioxin-like PCB can be expressed in a single number—the toxic equivalents (TEQ). This is defined by the sum of the products of the concentration of each compound (17 PCDD/PCDF congeners with 2,3,7,8-substitution and 12 dioxin-like PCB congeners) multiplied by their corresponding TEF value. This is an estimate of the total 2,3,7,8-TCDD-like toxicity of the mixture.

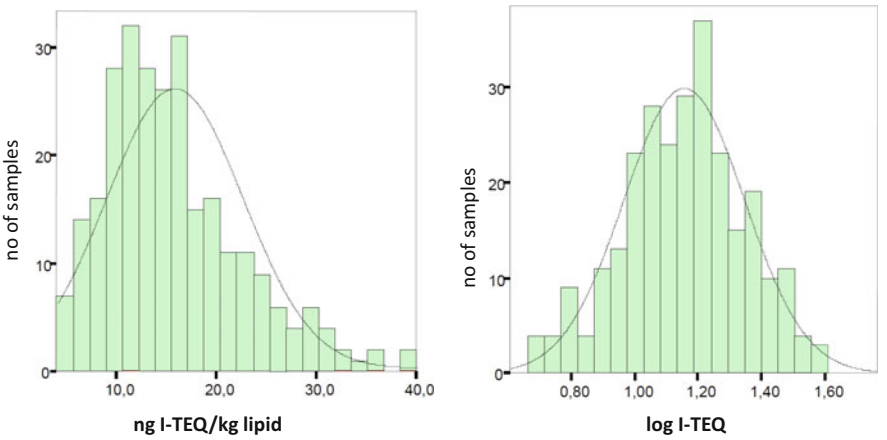

Fig. 1 Frequency distribution of 271 individual human milk samples collected in Germany from 1995 to 1998 (reprint from Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit [BMU] 2002, with permission from BMU)

Several institutions have derived different TEF schemes. When the first WHO-coordinated exposure study on levels of dioxins and dioxin-like PCB in human milk was performed (1987–1988), TEQ levels were calculated on the basis of TEF from US EPA and Nordic models (WHO 1989). Subsequently, TEQ levels for the second study (1992–1993) were calculated on the basis of International Toxic Equivalency Factors (I-TEF) and TEF proposed by WHO in 1994 (WHO 1996).

At an expert meeting held by the European Centre of Environmental Health of WHO (ECEH-WHO) and the International Programme on Chemical Safety (ICPS) in 1997, TEF values were re-evaluated. This resulted in a consensus on the WHO_{1998}-TEF (Van den Berg et al. 1998), which were widely used for many years, including the two WHO-coordinated studies covering the sampling periods from 2000 to 2003 and from 2004 to 2007.

Changes in WHO_{1998}-TEF values were proposed at another WHO expert meeting held in 2005 and resulted in WHO_{2005}-TEF (Van den Berg et al. 2006). The values of these WHO_{2005}-TEF have been validated since then and have been used for subsequent reports. Since 2011, EU regulations setting maximum limits for PCDD/PCDF and dioxin-like PCB in food and animal feed were amended to use WHO_{2005}-TEF for calculating maximum levels (European Commission 2011; European Commission 2012). The 2019 version of the "Guidance on the Global Monitoring Plan for persistent organic pollutants" mentions that according to the text of the Stockholm Convention (Annex C), the TEF as established by a WHO Expert Group and published in 1998, i.e., WHO_{1998}-TEF, should be used. However, state-of-the-art presentation of results would use the WHO_{2005}-TEFs and, therefore, it was recommended to report these as well in order to allow comparison with data from the literature and other reports (UNEP 2019).

Table 1 Changes of concentrations expressed as WHO-PCDD/PCDF-TEQ, WHO-PCB-TEQ, and WHO-PCDD/PCDF-PCB-TEQ, when WHO_{1998}-TEF are used instead of WHO_{2005}-TEF (as 100%: concentrations calculated as WHO_{2005}-TEQ) (232 samples)

	TEQ (WHO_{1998}-TEF) as % of TEQ (WHO_{2005}-TEF)					
	Minimum	25th percentile	Median	75th percentile	95th percentile	Maximum
WHO-PCDD/PCDF-TEQ	103%	112%	116%	120%	125%	132%
WHO-PCB-TEQ	92.2%	128%	145%	162%	195%	248%
WHO-PCDD/PCDF-PCB-TEQ	106%	118%	123%	134%	155%	191%

Table 1 presents the changes of WHO-TEQ-based results for all 232 samples if the former WHO_{1998}-TEF were applied instead of the current WHO_{2005}-TEF. The evaluation of the 232 data sets from all samples shows that concentrations calculated with WHO_{1998}-TEF as toxic equivalents of PCDD/PCDF (WHO_{1998}-PCDD/PCDF-TEQ) are on average about 16% higher (range about 3–32%) than for WHO_{2005}-PCDD/PCDF-TEQ. In particular, TEQ of dioxin-like PCB (WHO_{1998}-PCB-TEQ) were on average about 45% higher compared to the WHO_{2005}-PCB-TEQ results (range between about 10% lower and 150% higher). The total TEQ (WHO_{1998}-PCDD/PCDF-PCB-TEQ) were on average about 23% higher (range between 6 and 91%) than the corresponding WHO_{2005}-PCDD/PCDF-TEQ results. In general, this is in line with studies on human milk reporting a 20–25% decrease when WHO_{2005}-TEF are used instead of WHO_{1998}-TEF (Van den Berg et al. 2006; Wittsiepe et al. 2007).

In its recent assessment on the risk for human and animal health related to the presence of PCDD/PCDF and dioxin-like PCB in food and feed, the European Food Safety Authority (EFSA) re-evaluated its previous tolerable weekly intake for humans. As part of this re-evaluation, it was concluded that the current WHO_{2005}-TEF for the dioxin-like PCB 126 might be too high and a further discussion on all WHO_{2005}-TEF was proposed (EFSA, 2018). Therefore, the European Commission formally requested WHO to review the values for the WHO_{2005}-TEF. As conclusion, it is of general importance to always clarify which TEF values were applied for a reported TEQ value, e.g. as WHO_{1998}-TEQ or WHO_{2005}-TEQ. If data on the actual concentrations of the relevant congeners are available, re-calculation of the TEQ using modified TEF values is possible.

2.5 Lower and Upper Bounds TEQ Concentrations

Details on the calculation of toxic equivalents (TEQ) of mixtures of PCDD/PCDF and dioxin-like PCB using toxic equivalency factors (TEF) and on analytical criteria are given in the analytical chapter in Part II (Malisch and Schächtele 2023). An important criterion for assessment of the reliability of results is the difference between the lower-bound TEQ result (where non-detects = 0) and the upper-bound TEQ result (where non-detects = limit of quantification), as proposed as part of harmonized quality criteria for analyses of PCDD and PCDF (Malisch et al. 2001).

The 2019 version of the document 'Guidance on the Global Monitoring Plan for persistent organic pollutants' recommends that this difference between the lower-bound and upper-bound concentrations should be reported. As a measure of analytical quality assurance and quality control (QA/QC), this difference should be less than 20% (UNEP 2019).

The acceptable difference between lower- and upper-bound values is of particular importance for the analysis of samples intended to be used as a control of time trends for the effectiveness evaluation of the Stockholm Convention. If the difference is too high, changes of WHO-TEQ levels might be actually caused by changes of the analytical sensitivity and not by changes of the real levels of POPs in samples. In particular, samples with limited amounts or samples with low fat levels are at considerable risk of having a high difference between lower- and upper-bound WHO-TEQ levels. Therefore, regardless of whether human milk, human blood, air, or other matrices are analysed, all studies intended to be used for the effectiveness evaluation of the Stockholm Convention should report lower- and upper-bound WHO-TEQ levels that are within the acceptable range.

One of the features of the WHO/UNEP protocol is the collection of 50 ml individual human milk samples, which is relatively easy and non-invasive. With a lipid content of about 4% and preparation of a pooled sample of 50 individuals, a sufficient amount of sample is available to apply different analytical methods for determination of all 30 POPs presently listed in the Stockholm Convention and to assure that QA/QC criteria are met, including acceptable differences between lower- and upper-bound values for PCDD/PCDF analysis.

In contrast, human blood has a number of sampling difficulties as well as considerably lower lipid content. Therefore, meeting the requirement of an acceptable difference between lower- and upper-bound WHO_{2005}-TEQ levels takes considerably more effort for blood samples.

Table 2 summarizes the differences (in %) between lower and upper bounds for total TEQ concentrations of PCDD/PCDF and dioxin-like PCB (WHO_{2005}-TEQ) in all 232 samples. In particular, all samples fulfilled the QA/QC criterion with 98% of all samples having differences below 1%, which is considered negligible. Therefore, only the upper-bound WHO_{2005}-TEQ levels are used for discussion of the results.

Table 2 Differences (in %) between lower- and upper-bound total TEQ concentrations of PCDD/PCDF and dioxin-like PCB (WHO_{2005}-TEQ)

	No of samples	Min	25th percentile	Median	Mean	90th percentile	95th percentile	98th percentile	Max
Differences (in %) between lower- and upper-bound WHO_{2005}-TEQ levels	232	0	0	0.03	0.15	0.14	0.20	0.46	15.3

2.6 Non-dioxin-like PCB

Concentrations of non-dioxin-like PCB are expressed as the sum of six Indicator PCB (ΣPCB_6) including the congeners number 28, 52, 101, 138, 153, and 180. These are major marker congeners of the technical PCB mixtures (Schulte and Malisch 1983; Takasuga et al. 2006). Their sum usually comprises about half of the amount of total non-dioxin-like PCB present in feed, food, and humans and is considered to be an appropriate marker for occurrence in food and for human exposure to non-dioxin-like PCB. Therefore, this sum has been used in EU legislation since 2011 for setting maximum levels for non-dioxin-like PCB in food (European Commission 2011) and feed (European Commission 2012). Through biomagnification in the food chain, certain PCB, including PCB 28, PCB 52, and PCB 101, can be metabolized, e.g., by cows and finally by humans. As a result, PCB 138, PCB 153, and PCB 180 contribute on average about 50% in butter fat or in lipids in raw milk and about 60% to the sum of the individual concentrations of PCB in human milk (Schulte and Malisch 1984; Malisch and Schulte 1985; Kypke-Hutter and Malisch 1989).

Initially, the sum of these six congeners plus PCB 118 (ΣPCB_7) was used by UNEP (UNEP 2007). However, the mono-ortho PCB 118 also has dioxin-like properties and is included in the TEQ calculations as well (Van den Berg et al. 2006). As no chemical should be reported or regulated twice, since 2013 the revised "Guidance document on the Global Monitoring Plan for persistent organic pollutants" uses also the sum of six Indicator PCB (ΣPCB_6) (UNEP 2013b, 2019). As guidance for the differences between ΣPCB_6 and ΣPCB_7, the concentrations of PCB 118 contribute about 10% (calculated as median; range 2–30%) to the sum of 7 Indicator PCB based on the evaluation of all 232 samples received.

2.7 Use of Terms for TEQ

A complete and unambiguous system for concentrations expressed in terms of TEQ is needed to indicate which specific TEF is used (WHO_{1998}-TEF, or WHO_{2005}-TEF) and whether the calculation is the lower bound (LB) or upper bound (UB). European Union legislation uses the notation "WHO-PCDD/F-TEQ" for PCDD and PCDF, "WHO-PCB-TEQ" for dioxin-like PCB, and "WHO-PCDD/F-PCB-TEQ" for total TEQ to specify maximum levels of these contaminants in feed and food, with the definition that WHO_{2005}-TEFs are applied and upper-bound results are used. Without this separate definition, an unambiguous term for total TEQ would be, e.g., "WHO-PCDD/PCDF-PCB-TEQ (2005, UB)" or "WHO-PCDD/PCDF-PCB-TEQ (WHO_{2005}-TEF, UB)".

However, this system considerably reduces the readability. Though inaccurately, PCDD are sometimes shortly called "dioxins", and PCDF "furans". Moreover, the term 'dioxins' is commonly used also to refer to both PCDD and PCDF and is therefore quite ambiguous. For the sake of clarity in the following sections, the term PCDD/PCDF will be used consistently to refer to this group of compounds. In the text, "Total TEQ of PCDD/PCDF and dioxin-like PCB" or "WHO_{2005}-TEQ" is used comprising PCDD/PCDF and dioxin-like PCB. "TEQ of PCDD/PCDF" is used for "WHO-PCDD/PCDF-TEQ" and "TEQ of dioxin-like PCB" for "WHO-PCB-TEQ".

Tables and figures will employ the explicit EU terminology to avoid confusion if the tables and figures are taken out of context. Unless otherwise stated, all TEQ concentrations are based on calculations applying the WHO_{2005}-TEF and are calculated as upper-bound (UB) concentrations.

All concentrations for PCDD/PCDF and PCB are reported on lipid basis.

2.8 Human Exposure and Congener Patterns

While accidental and occupational dioxin exposure is normally limited to more or less small subgroups of the population, environmental exposure due to diffuse sources affects all humans. In comparison to other exposure routes (inhalation of air; ingestion of soil; dermal absorption), more than 90% of human dioxin exposure derives from food. Of this, about 90% normally comes from food of animal origin. Contamination of food is primarily caused by release of dioxins from various sources (e.g. waste incineration, production of chemicals, metal industry), and their subsequent accumulation in the food chain where they are particularly associated with fat (Fürst et al. 1992; European Commission—Scientific Committee on Food 2001a). The total global PCDD/PCDF release from 196 countries/regions was estimated to be 100.4 kg TEQ/year. Reference years were between 1998 and 2011, with the period 2000–2005 for about 90% of the countries (Wang et al. 2016).

Congener patterns of PCDD/PCDF and PCB differ between sources and are important tools for source identification. The review of congener patterns of PCDD/PCDF and PCB as useful aid to source identification during a contamination incident in the food chain (Hoogenboom et al. 2020), the review of the relevance of dioxin and PCB sources for food from animal origin (Weber et al. 2018), and the review of the investigative work necessary to find the source of contamination in incidents with dioxins and PCB in feed and food (Malisch 2017) might be helpful to find sources and to reduce exposure.

3 Overall Comparison of Concentrations of TEQ and Non-dioxin-like PCB Among UN Regions

A suitable starting point for the discussion of the complex picture is the comparison of the results for the most important sum parameters among UN regions, namely: (1) total TEQ of PCDD/PCDF and dioxin-like PCB, with further differentiation between TEQ of PCDD/PCDF and TEQ of dioxin-like PCB, and (2) Indicator PCB, calculated as ΣPCB_6.

3.1 TEQ

The range of concentrations of total TEQ in 232 samples from 82 countries collected between 2000 and 2019 varies between 1.29 and 49 pg WHO_{2005}-TEQ/g, with a median of 7.24 pg/g (Table 3; Fig. 2). The highest median WHO_{2005}-TEQ

Table 3 Range of concentrations of total TEQ (WHO_{2005}-TEQ), WHO-PCDD/PCDF-TEQ, and WHO-PCB-TEQ among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; pg/g lipid; $N = 232$)

UN Regional Group	No of countries	No of samples	WHO_{2005}-TEQ			WHO-PCDD/PCDF-TEQ			WHO-PCB-TEQ		
			Min	Median	Max	Min	Median	Max	Min	Median	Max
African	19	40	1.29	5.79	49.0	1.01	3.14	41.2	0.27	1.95	7.79
Asia-Pacific/Asia[a]	12	29	2.38	8.55	26.7	1.80	6.06	22.2	0.58	3.05	4.51
Asia-Pacific/Pacific	10	24	1.76	4.06	11.6	1.29	2.63	9.32	0.47	1.23	2.62
Latin American and Caribbean	14	36	1.81	4.68	10.4	1.26	3.41	8.44	0.55	1.16	4.95
Eastern European	11	43	4.10	12.0	24.3	2.40	6.04	10.7	1.53	5.71	13.6
Western European and "Others"[b]	16	60	3.85	10.3	26.9	2.68	6.72	18.6	1.09	3.60	11.3
All Regional Groups	82	232	1.29	7.24	49.0	1.01	4.65	41.2	0.27	2.57	13.6
Non-European countries (Asia, Pacific, Latin American and Caribbean, "Others"[b]	58	140	1.29	5.38	49.0	1.01	3.48	41.2	0.27	1.62	7.79
European countries[a] (Eastern and Western except "Others"[b])	24	92	4.10	12.0	26.9	2.40	6.50	18.6	1.39	5.00	13.6

[a]Including Cyprus
[b]"Others": Australia, Israel, New Zealand, USA (see Sect. 2.1)

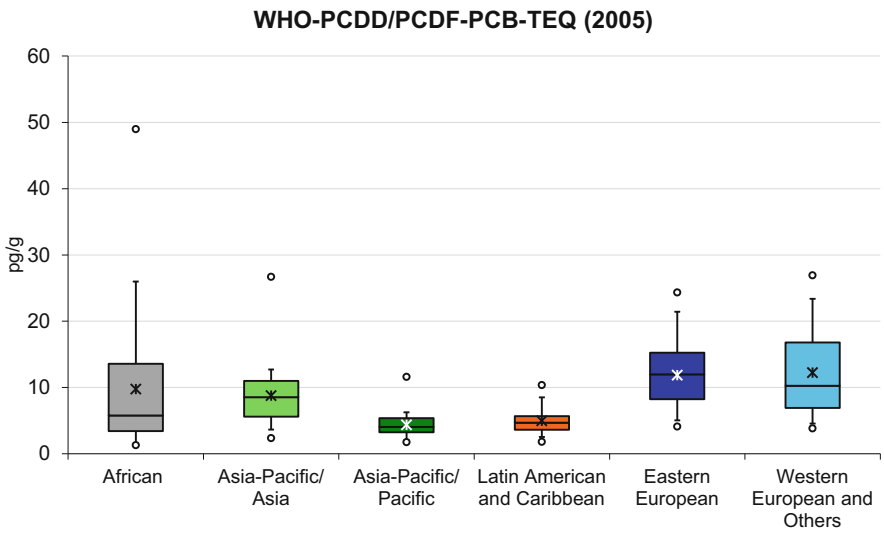

Fig. 2 Range of concentrations of total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; pg/g lipid, upper bound; $N = 232$) [box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

concentrations were found in countries of the Eastern European Group and the Western European and Others Group with 12.0 pg/g and 10.3 pg/g, respectively. The widest variation was in Africa (range 1.29–49 pg/g). With median concentrations between 4 and 5 pg/g and maximum levels between 10 and 12 pg/g, the Pacific region in the Asia-Pacific Group and countries from the Latin American and Caribbean Group were at the lower end of the distribution.

A closer look reveals that countries of the Eastern European Group and the Western European and Others Group have a higher contribution of dioxin-like PCB to the total TEQ than countries from other regions. As the Western European and Others Group comprises also Australia, Israel, New Zealand, and USA and the Asian Group also Cyprus, Table 3 includes also a differentiation between the 24 European countries and the 58 Non-European countries. Whereas in European countries the contribution of the median of dioxin-like PCB to the total TEQ is about 42%, this is in Non-European countries about 30%.

3.2 Non-dioxin-like PCB

The range of concentrations of the sum of 6 indicator PCB (ΣPCB_6) varies between approximately 1 and 1000 ng/g lipid, with a median of about 30 ng/g lipid (Table 4, Fig. 3). The highest concentrations were found in the Eastern European Group (median of about 120 ng/g lipid and maximum of about 1000 ng/g lipid), followed

Table 4 Median and range of concentrations of the sum of 6 Indicator PCB (ΣPCB_6) among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; ng/g lipid; $N = 232$)

Regional Group	No of countries	No of samples	Sum 6 indicator PCB (ΣPCB_6)		
			Minimum	Median	Maximum
African	19	40	0.90	22.3	90.3
Asia-Pacific/Asia[a]	12	29	3.25	22.2	79.8
Asia-Pacific/Pacific	10	24	2.55	8.3	23.4
Latin American and Caribbean	14	36	3.01	15.8	96.5
Eastern European	11	43	14.6	121	1009
Western European and "Others"[b]	16	60	12.0	74.6	467
All Regional Groups	82	232	0.90	31.7	1009
Non-European countries (Asia, Pacific, Latin American and Caribbean, "Others"[b]	58	140	0.90	16.4	96.5
European countries[a] (Eastern and Western except "Others"[b])	24	92	14.6	118	1009

[a]Including Cyprus
[b]"Others": Australia, Israel, New Zealand, USA (see Sect. 2.1)

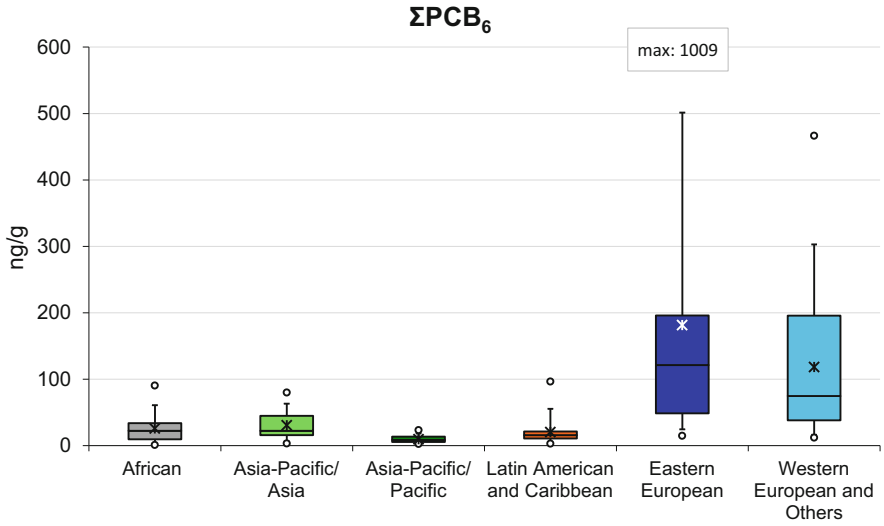

Fig. 3 Median and range and of levels of 6 Indicator PCB (ΣPCB_6) among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; ng/g lipid, upper bound; $N = 232$) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

by the group of Western European and Other States (median about 75 ng/g lipid, maximum 467 ng/g lipid). In all other groups, considerably lower PCB levels were found (maximum lower than 100 ng/g lipid, median approximately between 8 and 22 ng/g).

With regard to the above-mentioned inclusion of Australia, Israel, New Zealand, and USA in the Western European and Others Group and of Cyprus in the Asian Group, Table 4 includes the differentiation between the 24 European countries (median 118 ng/g lipid, range about 15–1000 ng/g) and the 58 Non-European countries (median 16 ng/g lipid, range about 1–100 ng/g). This clearly supports the conclusion that PCB concentrations are considerably higher in Europe than in the other geographic regions.

4 The Five WHO- and UNEP-Coordinated Studies from 2000 to 2019

As a first step in differentiation, results of the five studies are compared "round by round" in chronological order. As the five studies performed over 20 years had different time lengths, it is considered more appropriate to present the participation of countries in five equal rounds of 4 year each, namely: 2000–2003, 2004–2007, 2008–2011, 2012–2015, and 2016–2019 (Malisch et al. 2023a).

Following the protocol that was in effect, most countries of the 2000–2003 round submitted two or more pooled samples, with the option to add pooled samples from exposure groups expected to be high compared to the exposure group considered to be representative for the country. In subsequent rounds, most countries submitted only one pooled sample, which was considered as representative of the country, and the option to include pooled samples from expected high exposure groups was discontinued. In order to represent one country in each period by one result as "country results" in some summarizing figures, aggregated data based on the median levels were derived if a country submitted two or more pooled samples in a certain period (see Sect. 2.2).

For each of these rounds, the most important statistical data (minimum, median, and maximum) for the most important sum parameters, namely the toxic equivalents of PCDD and PCDF (WHO-PCDD/PCDF-TEQ), dioxin-like PCB (WHO-PCB-TEQ) and the total TEQ (WHO_{2005}-TEQ) and the sum of 6 Indicator PCB (ΣPCB_6), are compiled in Table 5 with regard to (1) the country results (1 result/country) and (2) the individual 232 pooled samples. The results in this table show that for all rounds, the median of the country results with aggregated data and that of single pooled samples for TEQ-based results are rather comparable: The country results with aggregated data for **TEQ of PCDD and PCDF** range from 1.01 to 22.2 pg/g with a median of 3.81 pg/g, for **TEQ of dioxin-like PCB** from 0.27 to 10.7 pg/g with a median of 1.86 pg/g, and for **total TEQ** from 1.29 to 26.7 pg/g with a median of 5.69 pg/g. The highest concentrations in the single pooled samples were 41.2 pg/g for TEQ from PCDD and PCDF, 13.6 pg/g for TEQ from dioxin-like PCB, and 49.0 pg/g for total TEQ, all found in the 2000–2003 round.

The country results with aggregated data for **ΣPCB_6** range from 0.90 to 502 ng/g with a median of 23.6 ng/g. At the 2000–2003 round, the optional inclusion of samples from expected high exposure groups might have caused the observed differences between the median of 123 ng/g for aggregated data (with aggregation

Table 5 Median and range of concentrations of (1) TEQ of PCDD and PCDF (WHO_{2005}-PCDD/PCDF-TEQ), dioxin-like PCB (WHO_{2005}-PCB-TEQ), and total TEQ (WHO_{2005}-PCDD/PCDF-PCB-TEQ) and (2) ΣPCB_6 in the five rounds performed between 2000 and 2019 with differentiation between country results with aggregated data (1 result/country with use of median or if 2 or more samples were submitted by a country, the median) and all single pooled samples

			WHO-PCDD/F-TEQ (2005/UB)	WHO-PCDD/F-TEQ (2005/UB)	WHO-PCB-TEQ (2005/UB)	WHO-PCB-TEQ (2005/UB)	WHO-PCDD/F-PCB-TEQ (2005/UB)	WHO-PCDD/F-PCB-TEQ (2005/UB)	Sum 6 Indicator PCB	Sum 6 Indicator PCB
			1 result/country (median, if 2 or more samples)	Single pooled samples	1 result/country (median, if 2 or more samples)	Single pooled samples	1 result/country (median, if 2 or more samples)	Single pooled samples	1 result/country (median, if 2 or more samples)	Single pooled samples
			pg/g lipid	pg/g lipid	pg/g lipid	pg/g lipid	pg/g lipid	pg/g lipid	ng/g lipid	ng/g lipid
Period	2000–2003	Min	3.08	2.46	1.16	0.96	4.42	3.53	16.4	9.94
No of countries	26	Median	7.34	7.58	4.93	4.70	12.4	12.5	123	72.2
No of samples	102	Max	18.03	41.18	10.7	13.6	23.0	49.0	502	1009
Period	2004–2007	Min	2.94	2.94	1.22	1.07	5.06	4.75	10.1	8.61
No of countries	13	Median	4.83	4.73	2.62	2.59	6.93	6.87	49.2	42.7
No of samples	16	Max	8.93	8.93	6.96	6.96	15.7	15.7	376	376
Period	2008–2011	Min	1.31	1.31	0.70	0.70	2.01	2.01	4.05	4.05
No of countries	45	Median	3.81	3.91	1.86	1.93	5.62	5.67	18.1	17.2
No of samples	50	Max	22.2	22.2	7.47	7.47	26.7	26.7	78.9	78.9

Period	2012–2015	Min	1.01	1.01	0.53	0.53	1.54	1.54	2.15	2.15
No of countries	17	Median	3.81	3.27	2.47	2.19	6.30	5.70	24.1	22.5
No of samples	20	Max	8.61	8.61	4.72	4.72	11.1	11.1	158	158
Period	2016–2019	Min	1.02	1.02	0.27	0.27	1.29	1.29	0.90	0.90
No of countries	43	Median	2.62	2.63	1.00	1.00	3.88	3.90	12.7	13.2
No of samples	44	Max	9.97	9.97	3.70	3.70	11.6	11.6	109	109
Period	2000–2019	Min	1.01	1.01	0.27	0.27	1.29	1.29	0.90	0.90
No of countries	144	Median	3.81	4.64	1.86	2.62	5.69	7.32	23.6	31.7
No of samples	232	Max	22.2	41.2	10.7	13.6	26.7	49.0	502	1009

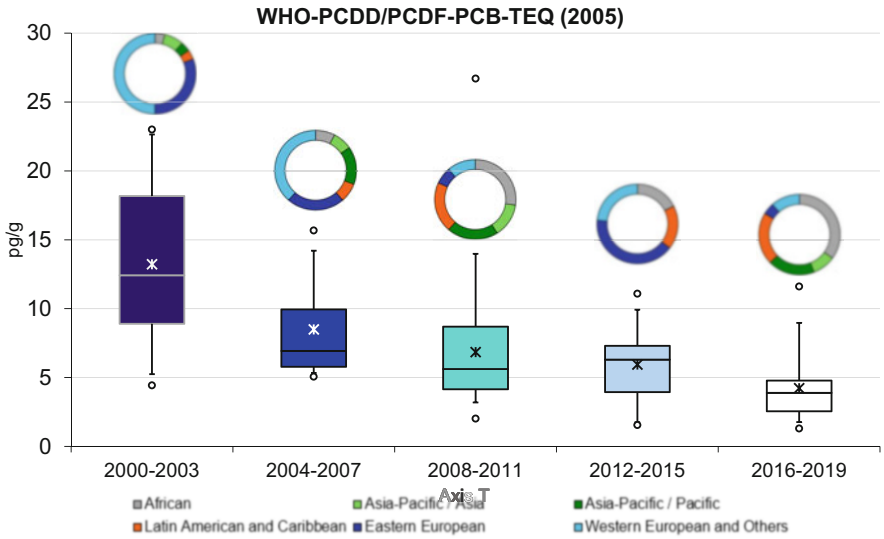

Fig. 4 Median and range of concentrations of total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in the five rounds performed between 2000 and 2019 and fraction of results among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; country results with aggregated data; pg/g lipid) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

for 25 of 26 countries) and the median of 72.2 for 102 single pooled samples. The highest concentration in the single pooled samples was 1009 ng/g for the sum of 6 Indicator PCB in the 2000–2003 period.

This compilation of data can be used for a general estimation of time trends. In Fig. 4, box plots illustrate the time trends over the five rounds: Over these 20 years, the median and range of **total TEQ** concentrations of the country results with aggregated data found in these five rounds went gradually down from initially 12.4 pg/g as median (range from 4.4 to 23.0) in the period 2000–2003 to 3.9 pg/g (range 1.3–11.6) in the period 2016–2019—a reduction of the median concentrations by 69%. However, changes in the fraction of regional groups over these periods have to be taken into consideration indicated by coloured circles above the box plots. Whereas in the 2000–2003 period, the majority of participants came from countries of the Eastern European Group and Western European and Others Group, in the 2016–2019 round, the majority came from the African Group, followed by the Group of Latin American and Caribbean Countries and then the Asia-Pacific Group.

For each of the five rounds, the country-specific results for the total TEQ are depicted in Figs. 28, 29, 30, 31, and 32 (in the appendix) and differ between (1) period of these studies and (2) UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands) (Fig. 28: period 2000–2003, Fig. 29: 2004–2007, Fig. 30: 2008–2011, Fig. 31: 2012–2015, and Fig. 32: 2016–2019). These figures are normalized to 30 pg/g as maximum value allowing

a direct visual comparison of the TEQ concentrations among the different collection periods as an indication of time trends. If two or more pooled samples were submitted, error bars indicate the range of the single pooled samples (minimum and maximum) around the median.

In the third round (2000–2003), 21 of the 26 countries participating were from the Eastern European or Western European and Others Groups. In comparison to other regions, countries from these groups had quite high total TEQ concentrations, e.g. (as aggregated data) Netherlands 22.8 pg/g (2001), Belgium 22.1 pg/g (2002), Luxembourg 21.7 pg/g (2002), Italy 20.3 pg/g (2001), Ukraine 19.2 pg/g (2002), and Germany 18.9 pg/g (2002). These countries were not all represented in the following rounds. However, if European countries participated, they were likely to be in the upper third or middle of the frequency distribution.

In the 2000–2003 round, Egypt had total TEQ concentrations comparable to European countries (23.0 pg/g in 2001; as aggregated data). In two African countries that were later found to be in the upper range of concentrations, i.e., Democratic Republic of Congo with 14.1 pg/g in 2009 and Côte d'Ivoire with 13.4 pg/g in 2010, a non-industrial source of contamination is assumed to have caused the elevated levels (see Sect. 5.1). The lower range of the frequency distribution curve for total TEQ in the period 2000–2008 was in the range of 5 pg/g and found in Fiji (4.42 pg/g in 2002; 5.06 pg/g in 2006), Brazil (5.24 pg/g in 2001–2002), Philippines (5.27 pg/g in 2002), Haiti (5.51 pg/g in 2004), Cyprus (5.70 pg/g in 2006), and Hungary (5.77 pg/g in 2006).

In the period 2012–2019, the low end of the frequency distribution of total TEQ (below 2 pg/g) were Ethiopia (1.29 pg/g in 2019; 1.54 in 2012), Uganda (1.59 pg/g in 2018), Niue (1.76 pg/g in 2017), Haiti (1.81 pg/g in 2015), Zambia (1.83 pg/g in 2019), and Vanuatu (1.95 pg/g in 2018). With concentrations between 6.7 and 11.6 pg/g, four countries from the African Group (Morocco, Senegal, Egypt, and Democratic Republic of Congo) and the Marshall Islands are found at the upper end of the frequency distribution curve of the 2016–2019 round.

For the sum of the 6 Indicator PCB ($\mathbf{\Sigma PCB_6}$), the country results with aggregated data are illustrated in Fig. 5. The highest concentrations by far were found in the period 2000–2003 with a median 123 ng/g (range 16–502), followed by the period 2004–2007 with a median 49 ng/g (range from 10 to 376 ng/g). In comparison, the other three rounds had considerably lower concentrations: The 2008–2011 round had a median of 18 ng/g (range from 4 to 79 ng/g); the 2012–2015 round had a median of 24 ng/g (range 2–158 ng/g); the 2016–2019 round had a median of 13 ng/g (range 1–109 ng/g). Thus, a considerable downward trend from the 2000–2003 round is observed to the period 2008–2011, obviously with a reduction by about 85% in the first decade of the 2000–2019 period, but the subsequent rounds seem to show a levelling out. However again, changes in the fraction of regional groups over these periods have to be taken into consideration. Whereas in the 2000–2003 period, the majority of participants came from European countries, which had higher PCB concentrations than other countries, in the 2016–2019 round, the majority came from Non-European countries. Therefore, this first indication of overall time trends needs to consider country-specific aspects, as well.

For each of the five rounds, the country results for the $\sum$ 6 Indicator PCB are depicted in Figs. 33, 34, 35, 36, and 37 (in the appendix) differentiating between:

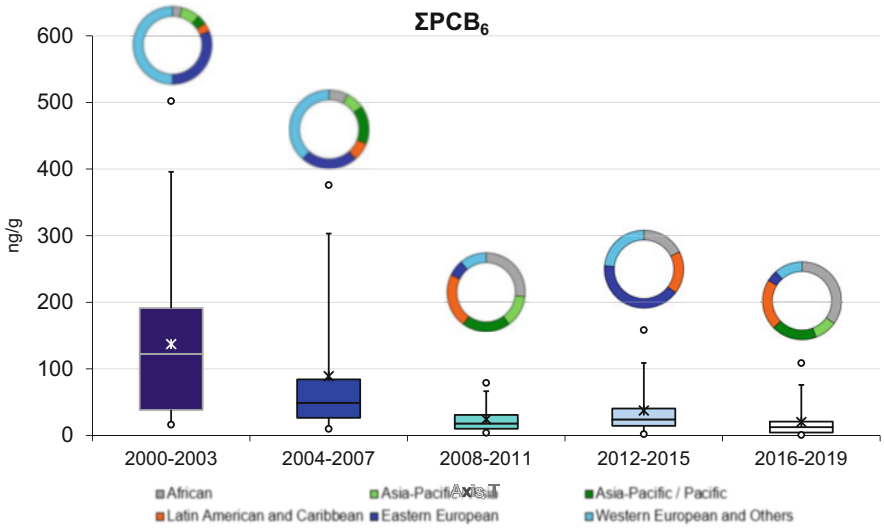

Fig. 5 Median and range of concentrations of the sum of 6 Indicator PCBs (ΣPCB_6) in the five rounds performed between 2000 and 2019 and fraction of results among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; country results with aggregated data; ng/g lipid) [box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the median: as box; mean: as asterisk]

(1) period of these rounds performed between 2000 and 2019 and (2) UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands). Figure 33 for the 2000–2003 round is normalized to 600 ng/g due to high levels, but the other results are normalized to lower levels. Note that the bold red line at the 100 ng/g level in all figures allows the downward trend over time to be easily observed. Following the principle of "one country – one result per round", the median is shown with error bars indicating the range of these samples (minimum and maximum), if a country submitted two or more pooled samples.

Between 2000 and 2003, the majority of participants were European countries. Later, WHO and UNEP encouraged countries of other groups to participate through special programmes. As shown in Sect. 3.2, European countries had by far the highest ΣPCB_6 concentrations. This is supported by the results of the 2000–2003 round, when only Eastern European and Western European and Others Regional Group countries were found in the upper third and middle part of the frequency distribution (Fig. 33). The 2004–2007 round also had considerably higher non-dioxin-like PCB concentrations in European countries (Fig. 34).

In these rounds, the highest ΣPCB_6 concentrations were found in samples from the Czech and the Slovak Republics. In one of the three pooled samples submitted by the Czech Republic in 2001, the highest concentration of 1009 ng/g was found. The median of the three submitted samples was 502 ng/g. However, the PCB concentrations in samples of these two countries decreased considerably to

109 ng/g in the Czech Republic and 78 ng/g in the Slovak Republic in the samples from 2019.

In the 2000–2003 round, other countries with elevated aggregated levels higher than 150 ng/g for ΣPCB_6 were Romania (173 ng/g in 2001), Belgium (191 ng/g in 2002), Netherlands (191 ng/g in 2001), Luxembourg (217 ng/g in 2002), Germany (220 ng/g in 2002), Spain (241 ng/g in 2002), and Italy (253 ng/g in 2001).

This estimation of time trends based on comparison of median concentrations in the five periods provides a first orientation. However, as the number of countries participating from a certain UN region in a certain period varies considerably, it can be influenced by the fraction of regions in a certain round or single results of a country submitted at a certain time. Thus, it is more precise to only use results of countries with repeated participation in these studies: This allows drawing conclusions on temporal trends, which are not potentially influenced by these possible factors. This assessment of time trends based only on the results of countries with repeated participation is published separately in Part IV (Malisch et al. 2023b).

5 Detailed Comparison of Concentrations on a Regional Group Scale

The Stockholm Convention's Global Monitoring Plan (GMP) for POPs is implemented in the five geopolitical United Nations regions, namely Africa, Asia and the Pacific, Eastern Europe, Latin America and the Caribbean, and Western Europe and Others. Countries that are party of the Convention are to report flexibly using one of these five UN Regional Groups. As the studies were performed since 2004 with the aim to contribute to the effectiveness evaluation of the Stockholm Convention, countries participating in WHO- and UNEP-coordinated studies have been classified by these five UN regions (Malisch et al. 2023a). The detailed results for all congeners and sum parameters are available at the GMP Data Warehouse (Global Monitoring Plan Data Warehouse 2020). In this section, the following figures illustrating the results of the 2000–2019 surveys for total TEQ and NDL-PCB in the regions were prepared by use of country results with aggregated data, if two or more samples were submitted (see Sect. 2.2).

5.1 African Group

Africa had the widest variation in contamination of human milk with **total TEQ** that was observed in any group. Figure 6 illustrates these results (with the five 4-year studies between 2000 and 2019 shown in different colours).

On one side was Ethiopia with the lowest levels of total TEQ of all countries in the 2000–2019 studies (1.54 pg WHO_{2005}-TEQ/g in 2012 and 1.29 pg/g in 2019). On the other side was Egypt with a median of 23.0 pg WHO_{2005}-TEQ/g for 9 pooled samples collected in 2001 and 2002, which are comparable to levels found in Europe at that time. With 49.0 pg WHO_{2005}-TEQ/g, one of the pooled samples from Egypt

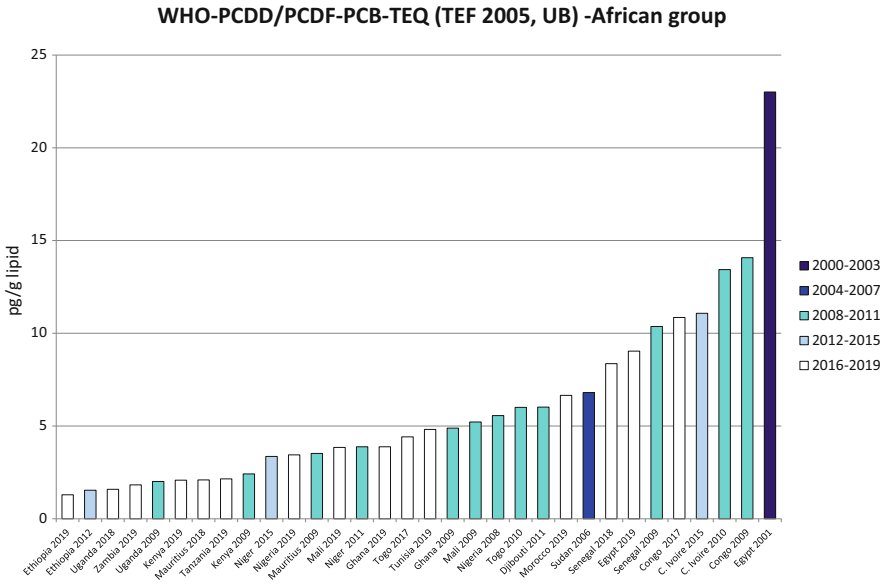

Fig. 6 Results of the 2000–2019 surveys for total TEQ in human milk in countries from Africa with indication of the period and year of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [TEF 2005, UB])/g lipid)

submitted in 2001 had a very high level of total TEQ and was probably from a contaminated area. However, the pooled sample from Egypt of 2019 (9.0 pg WHO_{2005}-TEQ/g) had considerably lower concentrations.

Uganda (2009 and 2018), Zambia (2019), Kenya (2009 and 2019), Mauritius (2018), and Tanzania (2019) were at the lower end of the frequency distribution among African countries (below 3 pg WHO_{2005}-TEQ/g). Niger (2011, 2015), Nigeria (2008, 2019), Mauritius (2009), Mali (2009, 2019), Ghana (2009, 2019), Togo (2010, 2017), Tunisia (2019), Djibouti (2011), Morocco (2019), and Sudan (2006) were in the middle (range 3–7 pg WHO_{2005}-TEQ/g). Senegal (2009, 2018), Côte d'Ivoire (2010 and 2015), Democratic Republic of Congo (2009, 2017) and, as mentioned, Egypt (2001, 2019) were in the upper third of the frequency distribution in the African Group (range 8–23 pg WHO_{2005}-TEQ/g).

Figure 38 (in the appendix) illustrates the relative contributions of toxic equivalency resulting from PCDD (WHO-PCDD-TEQ), PCDF (WHO-PCDF-TEQ), and dioxin-like PCB (WHO-PCB-TEQ) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ) in human milk in countries from Africa. Interestingly, the three countries with the highest levels of the total TEQ (Côte d'Ivoire [2010], Democratic Republic of Congo [2009], and Egypt [samples of 2001–2002]) were among the countries with low contributions from dioxin-like PCB (range 8–22%). Consistent with NDL-PCB concentrations between 10 and 50 ng/g lipid, this observation indicates that the elevated TEQ levels are not caused by a PCB contamination. However, whereas Egypt (2001) had the highest contribution to total TEQ from PCDF (41% from

WHO-PCDF-TEQ), Côte d'Ivoire (2010 and 2015) and Democratic Republic of Congo (2009 and 2017) had the highest contribution to total TEQ from PCDD (range 65–85% from WHO-PCDD-TEQ).

The PCDD-dominated patterns in human milk in the Democratic Republic of Congo and Côte d'Ivoire mirror with the patterns found in certain clays with high concentrations of PCDD/PCDF collected on the Dutch market originating from African countries and in trading centres in various African countries (Reeuwijk et al. 2013). Such patterns were found before, e.g. in the 1990s in clay from a mine in Mississippi causing a contamination of poultry and fish (Hayward et al. 1999) and later at contamination incidents in Germany and the Netherlands (reviewed by Malisch 2017). These congener patterns would be expected after bioaccumulation and hint at consumption of such clays ("geophagy") by pregnant women in these African countries as the likely source for these remarkably high levels in human milk (Hoogenboom et al. 2011, 2020; Reeuwijk et al. 2013; Malisch et al. 2011). It should be noted that with regard to the contribution to toxic equivalency, 1,2,3,7,8-PeCDD *(pentachlorodibenzo-p-dioxin)* is predominant in both samples from Côte d'Ivoire (2010, 2015), whereas in the Democratic Republic of Congo, 1,2,3,7,8,9-HxCDD *(hexachlorodibenzo-p-dioxin)* is by far the dominant congener in both samples (2009, 2017) (Fig. 7).

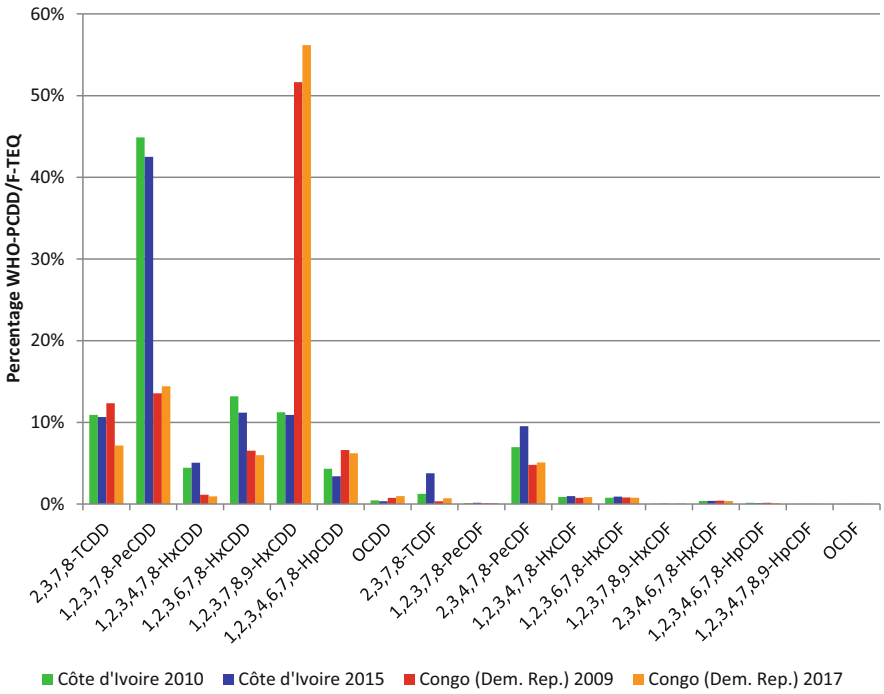

Fig. 7 PCDD/PCDF congener patterns in human milk in Côte d'Ivoire (2010, 2015) and Democratic Republic of Congo (2009, 2017)

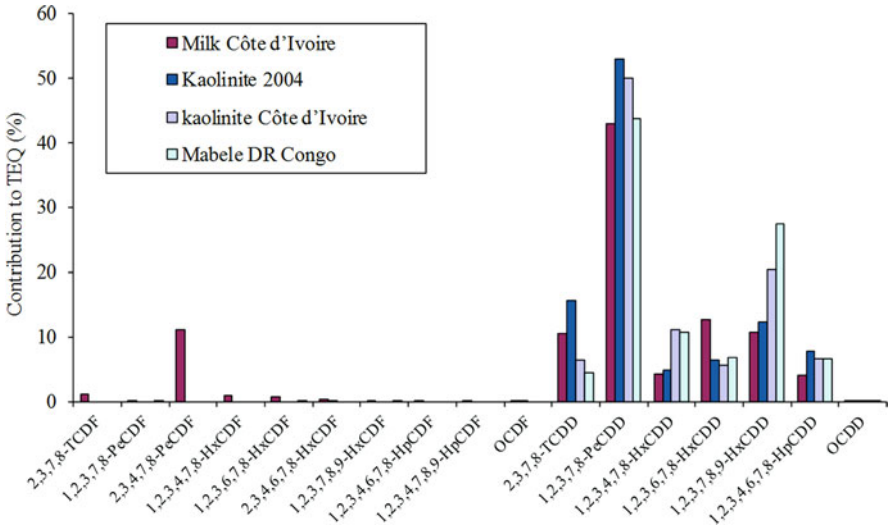

Fig. 8 Comparison of the congener patterns in human milk from Côte d'Ivoire (sample from 2010 for the WHO- and UNEP-coordinated exposure studies) with patterns observed in two Kaolinite and one Mabele clay samples (reprinted from Reeuwijk et al. 2013, with permission from Elsevier)

The pattern found in a kaolinic clay sample from Côte d'Ivoire was highly comparable with the kaolinic clay causing the 2004 contamination incident in the Netherlands. Also the pattern in the Mabele clay sample collected in the Netherlands and labelled "Democratic Republic of Congo" showed a similar pattern of congeners (Fig. 8, from Reeuwijk et al. 2013).

Figure 9 (from Reeuwijk et al. 2013) shows the congener pattern in samples of human milk from the Democratic Republic of Congo (sample from 2009) compared with three different clays that were all characterized by an usually high contribution of 1,2,3,7,8,9-HxCDD. In some clay samples, this congener showed the highest contribution to the TEQ-level (>60%). These clay patterns are remarkably similar to the patterns seen in human milk from the Democratic Republic of Congo and suggest that consumption of such contaminated clay is the reason for high PCDD/PCDF levels in human milk in this country.

Both the relatively high levels of PCDD in the human milk from Congo and Côte d'Ivoire collected for the fifth round (2008–2012) of the WHO- and UNEP-coordinated exposure studies and the similarity of the congener patterns with those from the clays, strongly suggest that the consumption of clays during pregnancy contributes to these high levels in human milk. Considering the susceptibility of the developing foetus and young children to PCDD/PCDF, the consumption of contaminated clays should be discouraged.

The mixture of a PCDF-dominated pattern with particularly high contribution of 2,3,4,7,8-PeCDF *(pentachlorodibenzofuran)* and PCDD to the TEQ levels as found in the nine human milk samples from Egypt (2000–2002) (Fig. 10) could indicate

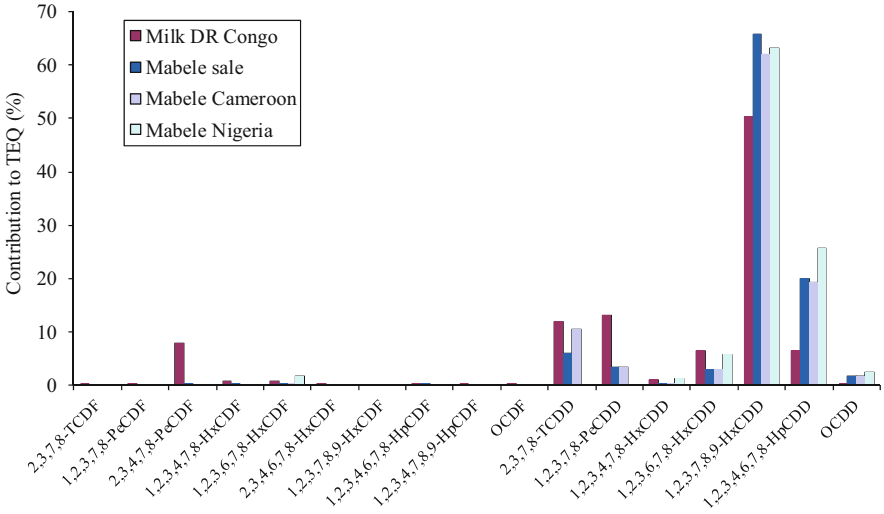

Fig. 9 Comparison of the congener pattern in human milk from the Democratic Republic of Congo (sample from 2009 for the WHO- and UNEP-coordinated exposure studies) with the patterns observed in three different Mabele clay samples with unusually high contribution of 1,2,3,7,8,9-HxCDD (reprinted from Reeuwijk et al. 2013, with permission from Elsevier)

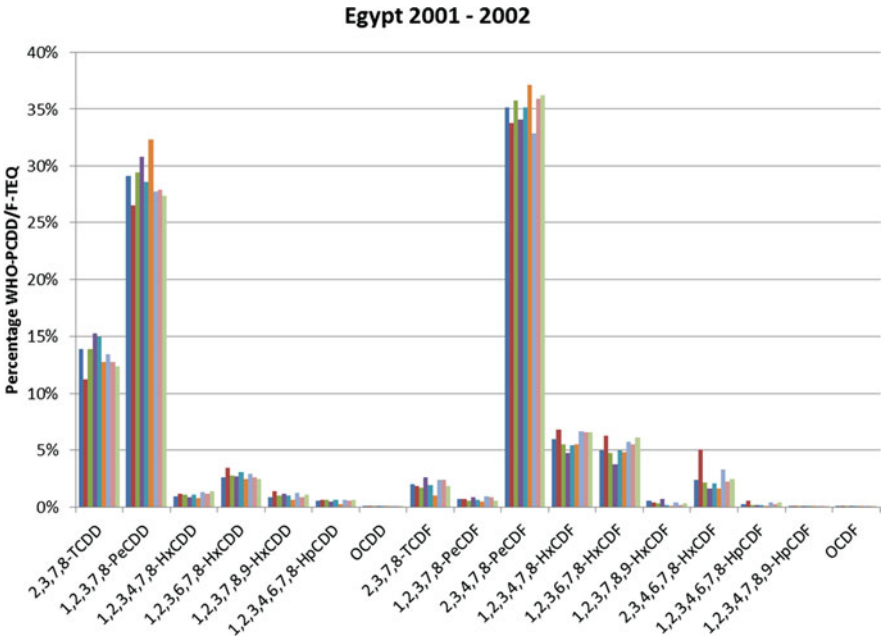

Fig. 10 Congener contributions (%) to toxic equivalency from PCDD and PCDF (WHO-PCDD/PCDF-TEQ) in human milk from Egypt (2001–2002)

combustion and drying processes as a source for this contamination (Hoogenboom et al. 2020). While Egypt covers an area of about 1,000,000 square kilometers, the great majority of its nearly 100 million people live along the banks of the Nile River with about half of the population living in urban areas. In this relatively small area, possible emission from industrial production as well as open burning of waste is in close proximity to agricultural production areas. This might explain the findings of high concentrations of PCDD and PCDF in foods in the 1990s, particularly in butter (Malisch and Saad 1994; Malisch and Saad 1996), and elevated levels of these contaminants in human milk collected in 1997 (Malisch et al. 2000).

The seven pooled samples collected in 2001 had a wide range (between 17.6 and 49.0 pg total TEQ/g). Note that these seven samples of 2001 were freeze-dried before shipment, and were apparently contaminated with lower chlorinated PCB during freeze-drying (Malisch et al. 2023b). Therefore, results for some PCB congeners are not useable. Hence, two additional pooled samples were submitted by Egypt in 2002, which were shipped frozen and not freeze-dried. These two samples had concentrations of 16.9 and 19.0 pg total TEQ/g lipid. In 2019, these levels decreased to 9.04 pg total TEQ/g lipid.

Figure 11 illustrates the results of the **sum of 6 Indicator PCB (ΣPCB_6)** with the period of participation between 2000 and 2019 indicated. Ethiopia also had the lowest levels of all countries in the 2000–2019 studies for this parameter: 2.15 ng ΣPCB_6/g lipid in 2012 and 0.90 ng/g in 2019. The highest level in Africa was found in Senegal where the sample of 2018 (90.3 ng/g lipid) showed an increasing trend in comparison to the sample of 2009 (65.8 ng/g lipid). This is at the upper end of the frequency distribution of all samples collected after 2010.

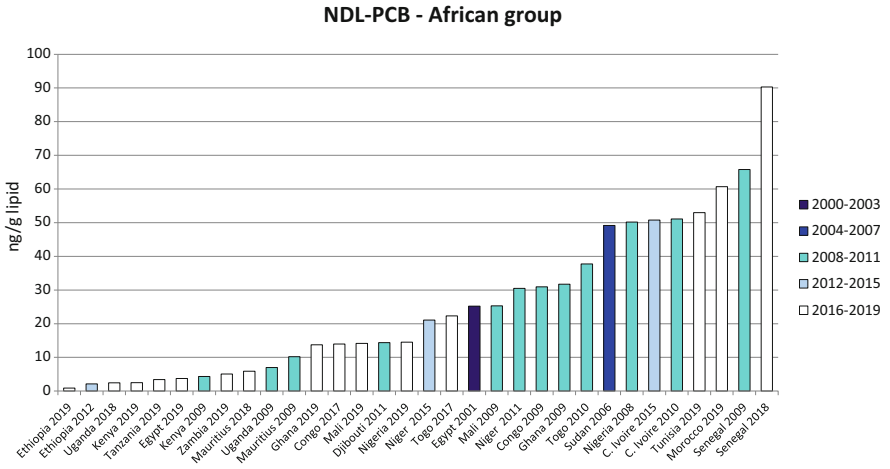

Fig. 11 Results of the 2000–2019 surveys for ΣPCB_6 (ng/g lipid) in human milk in countries from Africa with indication of the period and year of sample submission

5.2 Asia-Pacific Group

5.2.1 Asia Subgroup

Figure 12 illustrates the results of total TEQ with the period of participation between 2000 and 2019 indicated for the Asian countries of the Asia-Pacific Group. Concentrations below 5 pg WHO_{2005}-TEQ/g lipid were found in all samples of the 2016–2019 round (Thailand [2018], Mongolia [2018], Vietnam [2019], and Cambodia [2019]). Samples from previous rounds, with the exception of Syria (2009), had higher levels (UNEP 2013a). Hong Kong SAR of China participated twice, with a slight downward trend from the 2002 level of 10.8 pg total TEQ/g (median of 13 samples from different population subgroups [Hedley et al. 2006]) to the 2009 level of 9.4 pg/g total TEQ (median of 4 samples from different subgroups). Tajikistan (2009), Indonesia (2011), Cyprus (2006), Philippines (2002), and the Republic of Korea (2008) had concentrations between 5.2 and 7.3 pg/g lipid.

The lowest contribution of dioxin-like PCB to the total TEQ (14%) is found in Cambodia, the highest with 46% in Tajikistan. In Hong Kong SAR of China, the dioxin-like PCB contribution to TEQ was about 31 and 33% in 2002 and 2009, respectively (Fig. 39, in the appendix). The highest concentration of 46 ng ∑ 6 Indicator PCB/g found in Hong Kong SAR of China in 2002 was quite low in comparison to European countries at that time (see Fig. 33) and decreased to 22 ng/g in 2009 (Fig. 13).

5.2.2 Pacific Islands Subgroup

Figure 14 illustrates the results of total TEQ for samples from the Pacific Islands subgroup of the Asia-Pacific Group submitted between 2000 and 2019. All samples

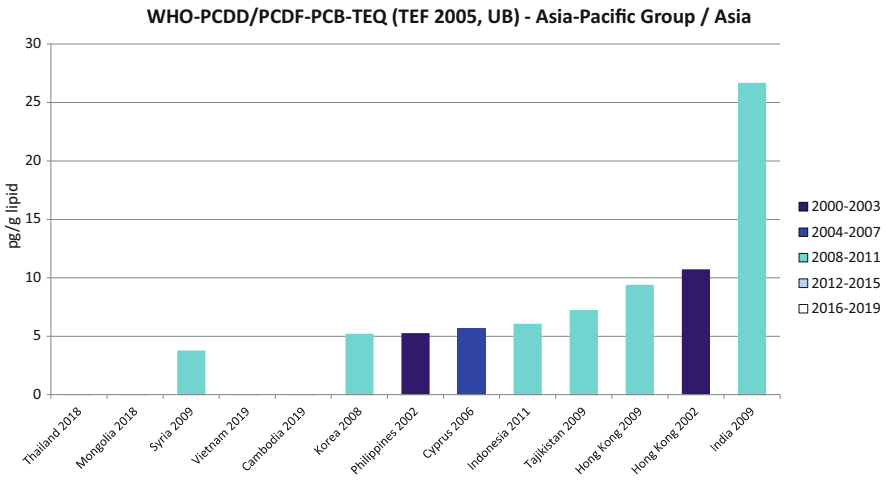

Fig. 12 Results of the 2000–2019 surveys for total TEQ in human milk from Asian countries of the Asia-Pacific Group with indication of the period and year of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [TEF 2005, UB])/g lipid)

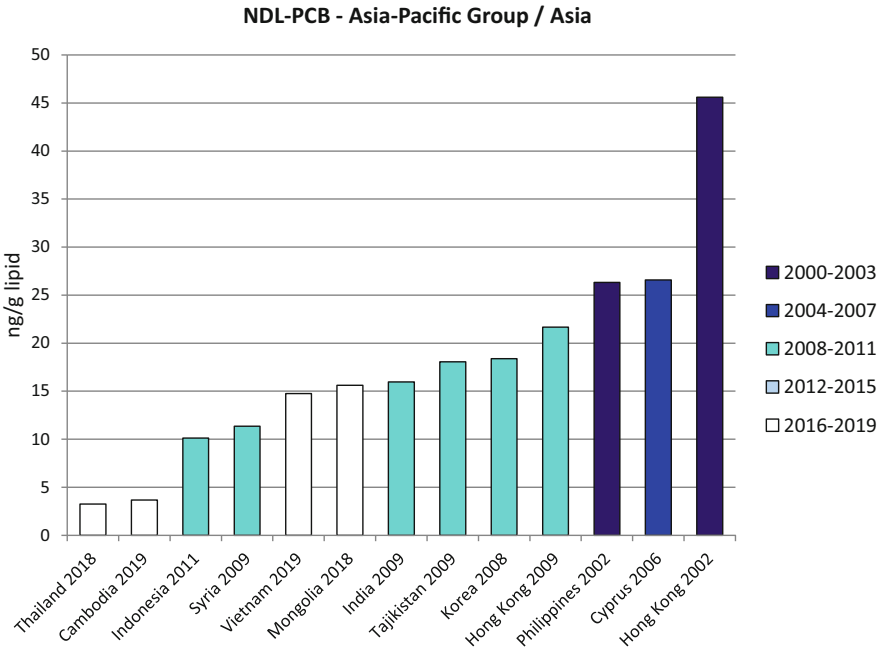

Fig. 13 Results of the 2000–2019 surveys for $\sum$ PCB_6 (ng/g lipid) in human milk from Asian countries of the Asia-Pacific Group with indication of the period and year of sample submission

submitted between 2000 and 2015 were approximately in the range 3–6 pg WHO_{2005}-TEQ/g lipid and included those from Tuvalu (2011), Fiji (2002, 2006, 2011), Solomon Islands (2011), Tonga (2008), Samoa (2011), Kiribati (2006, 2011), Niue (2011), and Palau (2011). In nearly all samples from the 2016 to 2019 period, concentrations were below 4 pg/g suggesting a downward trend, including samples from Niue (2017), Vanuatu (2018), Solomon Islands (2019), Fiji (2019), Kiribati (2018), Samoa (2019), and Palau (2018). Only one sample from the Marshall Islands (2019) had a substantially higher concentration of 11.6 pg/g, which was nearly double the 6.32 pg total TEQ/g lipid found in the sample from the Marshall Islands of 2011 and the highest concentration found in the Pacific Islands subgroup in the period 2000–2019.

With regard to the from 2011 to 2019 increasing concentration on Marshall Islands and the overall elevated concentration range of its two samples in the Pacific Islands subgroup, the changes of the relative contribution (%) of PCDD, PCDF, and dioxin-like PCB to the total toxic equivalents are of interest (Fig. 40, in the appendix). 73% contribution of PCDD to total TEQ in the sample from 2019 is the highest found in the Asia-Pacific Group. In this sample, 7% came from PCDF and 20% from dioxin-like PCB. This is a considerable change in comparison to its 2011 sample, when 40% of the total TEQ came from PCDD, 23% from PCDF, and 37% from dioxin-like PCB.

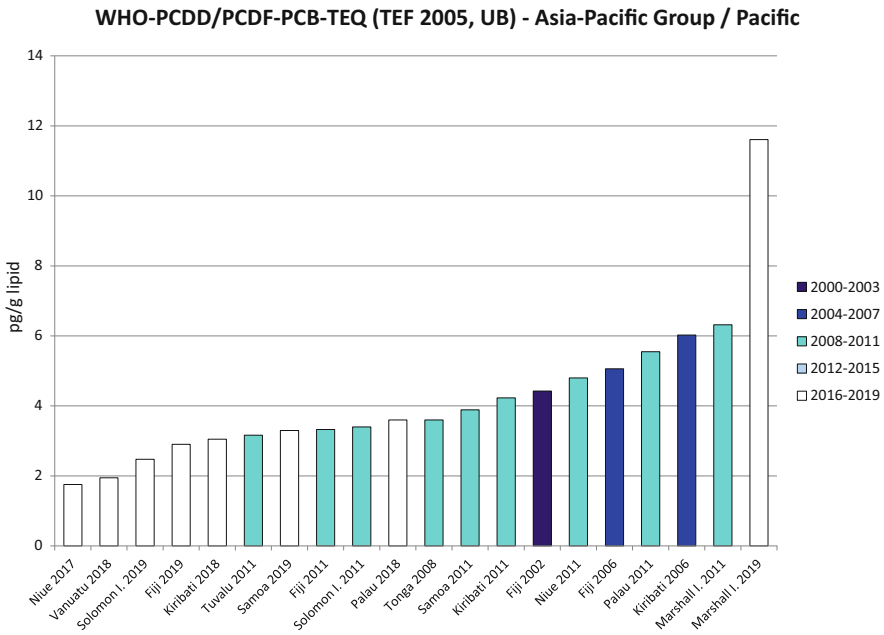

Fig. 14 Results of the 2000–2019 surveys for total TEQ in human milk from countries of the Pacific Islands subgroup in the Asia-Pacific Group with indication of the period and year of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [TEF-2005, UB])/g lipid)

A look into the PCDD/PCDF pattern of the 2019 sample from the Marshall Islands reveals that 1,2,3,7,8-PeCDD contributed 53% to the TEQ of PCDD and PCDF, 2,3,7,8-TCDD 13% and 2,3,4,7,8-PeCDF 5%, whereas these were 33%, 14%, and 24%, respectively, for the 2011 sample (Fig. 15). The 2019 pattern is less influenced by a thermal source than by chlorophenol-related substances. As example, a technical product of the pesticide 2,4-D (dichlorophenoxy acetic acid) was found to have 1,2,3,7,8-PeCDD as by far dominant TEQ contributor; within the 2,3,7,8-substituted HexaCDD, 1,2,3,6,7,8-HexaCDD contributed more than the other congeners (Holt et al. 2010). However, with regard to the huge variety of chlorophenol-related substances and their different and over time changing production processes, the PCDD/PCDF patterns can vary not only between different substances, but might vary also for the same chemical depending on the production process.

Regarding concentrations for NDL-PCB, most samples collected between 2000 and 2015 were in the range of approximately 4–17 ng/g for $\sum PCB_6$, whereas most samples for the period 2016–2019 were in the range of approximately 3–9 ng/g $\sum PCB_6$. Only the Marshall Islands showed an increase from 16 to 23 ng/g $\sum PCB_6$ from 2011 to 2019 (Fig. 16).

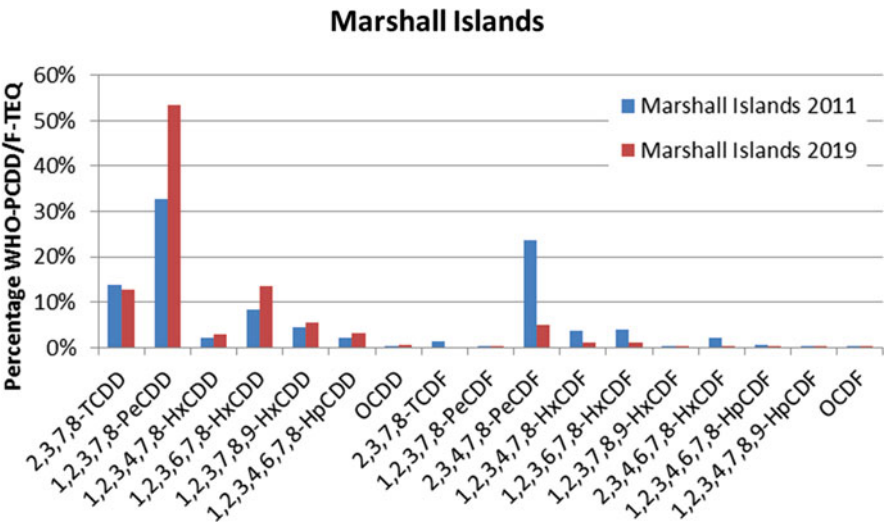

Fig. 15 Congeners contributions (%) from PCDD and PCDF to toxic equivalency (WHO-PCDD/PCDF-TEQ [2005]) in human milk from Marshall Islands in 2011 and 2019

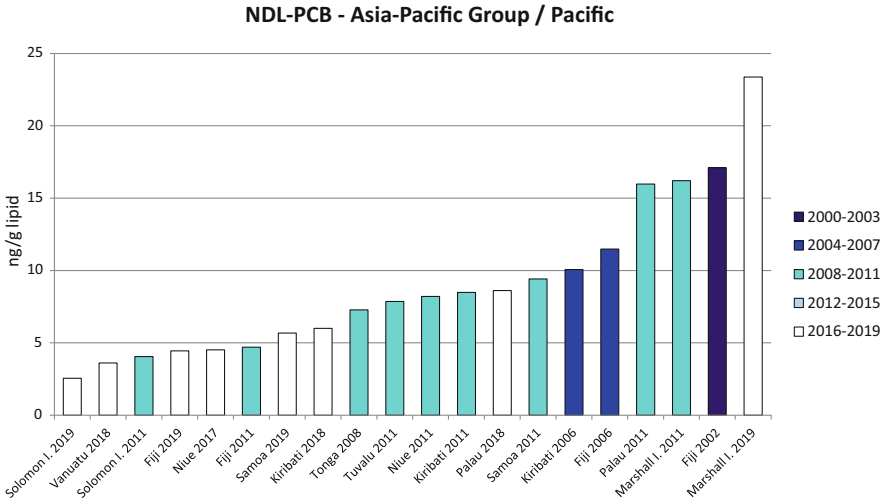

Fig. 16 Results of the 2000–2019 surveys for $\sum PCB_6$ (ng/g lipid) in human milk from countries of the Pacific Islands subgroup in the Asia-Pacific Group with indication of the period and year of sample submission

5.3 Group of Latin American and Caribbean Countries (GRULAC)

Figure 17 illustrates the results of total TEQ for the period 2000 and 2019 for countries from the Group of Latin American and Caribbean Countries. Note that for the period 2008–2011, Chile participated both in 2008 and in 2011. Also note

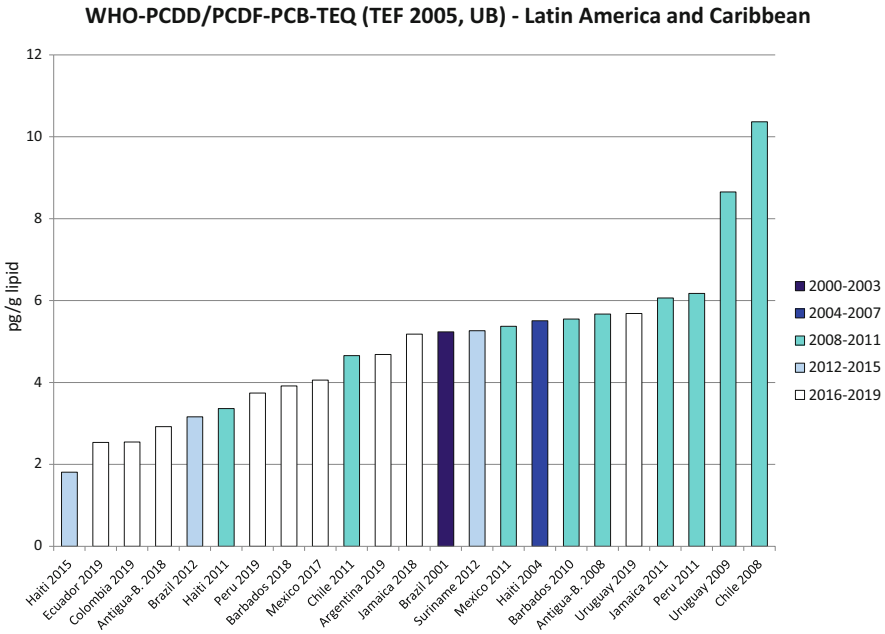

Fig. 17 Results of the 2000–2019 surveys for total TEQ in human milk samples from countries of the Group of Latin American and Caribbean Countries with indication of the period and year of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [TEF 2005, UB])/g lipid)

that the sample from Cuba (2011) was freeze-dried before shipment. As observed with freeze-dried samples from Egypt, a contamination with lower chlorinated PCB occurred during freeze-drying and resulted in an unusual PCB pattern (Malisch et al. 2023b). Therefore, results for dioxin-like PCB were not used in the calculation of WHO-PCB-TEQ and total TEQ in the Cuban sample.

Most samples of the period 2000–2011 were approximately in the range 3–6 pg total TEQ/g including samples from Antigua and Barbuda (2008), Barbados (2010), Brazil (2001), Chile (2011), Haiti (2004, 2011), Jamaica (2011), Mexico (2011), and Peru (2011). Uruguay (2009) and Chile (2008) were at the upper end with 8.65 and 10.4 pg total TEQ/g lipid, respectively. In the samples of the 2012–2019 period, total TEQ concentrations were found in the range from 1.8 to 5.7 pg/g suggesting a downward trend, which included samples from Antigua and Barbuda (2018), Argentina (2019), Barbados (2018), Brazil (2012), Colombia (2019), Ecuador (2019), Haiti (2015), Jamaica (2018), Mexico (2017), Peru (2019), Suriname (2012), and Uruguay (2019). With 3.44 pg WHO-PCDD/PCDF-TEQ/g lipid, Cuba (2011) was in the middle of the range of concentrations found for PCDD/PCDF in this group at that time, but, as noted above, this did not include a contribution of dioxin-like PCB.

Due to its huge size and population of over 200 million, Brazil submitted altogether 10 samples in 2001 and 2002 representing national and provincial areas

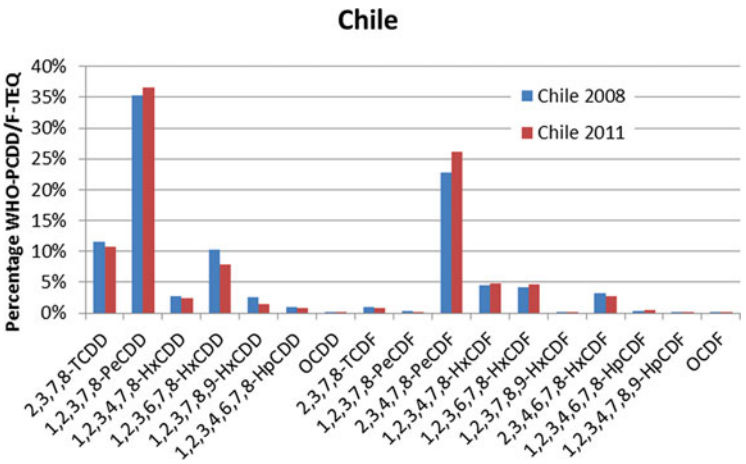

Fig. 18 Congener patterns (as contribution [%] of PCDD and PCDF to their toxic equivalency) in human milk samples from Chile in 2008 and 2011

(Braga et al. 2002). Total TEQ concentrations ranged between 3.53 and 8.47 pg/g with a median of 5.24 pg/g. Three national samples collected in 2012 had 3.00, 3.16, and 3.42 pg WHO_{2005}-TEQ/g lipid. Brazil is an example of the need for flexible criteria in collecting a representative sample for a country. According to the protocol, one pooled sample for countries with populations less than 50 million is requested. Countries with populations well over 50 million (or with sufficient resources) were encouraged to prepare a second pooled sample (or more) if feasible.

The relative contribution of PCDD, PCDF, and dioxin-like PCB to the total TEQ is depicted in Fig. 41 (in the appendix). The contribution of PCDD to the TEQ ranged from 30% (Peru [2011]) to 64% (Jamaica [2018]), for PCDF from 10% (Haiti [2004]) to 34% (Chile [2011]), and for dioxin-like PCB from 14% (Mexico [2011]) to 52% (Peru [2011]).

Figure 17 shows that the two samples from Chile were considerably different in total TEQ concentrations between 2008 (10.4 pg/g) and 2011 (4.7 pg/g). Neither the contribution of PCDD, PCDF, and dioxin-like PCB to the TEQ (Fig. 41) nor the PCDD/PCDF pattern (Fig. 18) changed during this relatively short time period of 3 years. Thus, differences in the regional origin of these two samples might explain these findings.

As the intake of PCDD/PCDF and PCB comes mainly from food (see Sect. 2.8), it might be of interest to understand the difficulties to find sources of contamination with the example of two incidents in the food chain in Chile. In 2008, the formation of PCDD/PCDF from a refinery process for zinc oxide used in feed additives was detected as source of a dioxin contamination in Chilean pork. PCDD/PCDF were formed at remarkably high concentrations in zinc oxide (17,147 pg TEQ/g) from a metal refinery process. 2,3,4,7,8-PeCDF contributed about 30% to TEQ concentrations. As follow-up of investigations of meat and associated supplies, in vegetal and animal fatty acid components more PCDD, especially 2,3,7,8-TCDD

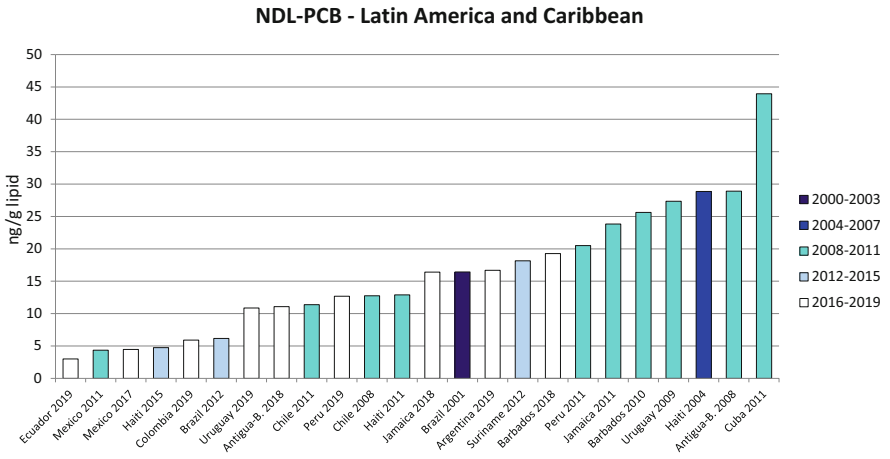

Fig. 19 Results of the 2000–2019 surveys for $\sum$ PCB_6 (ng/g) in human milk samples from countries of the Latin America and Caribbean Group with indication of the period and year of sample submission

and 1,2,3,7,8-PeCDD were found. This suggested that a secondary source of contamination may have existed in addition to zinc oxide, although the exact source could not be confirmed (Kim et al. 2011). As for the Marshall Islands, the kind of PCDD pattern in the secondary source is an indication for chlorophenol-related chemicals. Another incident occurred in Chile in 2013, when samples of chicken with elevated PCDD/PCDF concentrations showed a pattern not reported before. Certain non-2,3,7,8-PeCDD were present at much higher levels than 1,2,3,7,8-PeCDD. However, the actual source and site of the contamination was never discovered (Hoogenboom et al. 2020).

With regard to NDL-PCB in the Latin America and Caribbean Group, most samples collected between 2000 and 2011 were in the range of about 11–30 ng/g for $\sum PCB_6$ with a maximum of 44 ng/g in Cuba, whereas all samples of the period 2012–2019 were in the range of approximately 3–20 ng/g for $\sum$ PCB_6 (Fig. 19).

5.4 Eastern European Group

Figure 20 illustrates the results of total TEQ for the period between 2000 and 2019 for countries of the Eastern European Group. The eight countries submitting samples in the period 2000–2004 had concentrations that ranged between 8.18 pg WHO-PCDD/PCDF-PCB-TEQ/g for Hungary (2001) and 19.2 pg/g for Ukraine (2001). The samples collected between 2004 and 2011 were in the range between 5.8 pg/g (Hungary [2006]) and 15 pg/g (Modova [2009]). The trend to lower concentrations continued during the period 2012–2019, with the lowest concentrations being between 4 and 5 pg/g found in Croatia (2014), Czech Republic (2019), and Slovak Republic (2019). Samples from Bulgaria (2014), Czech Republic

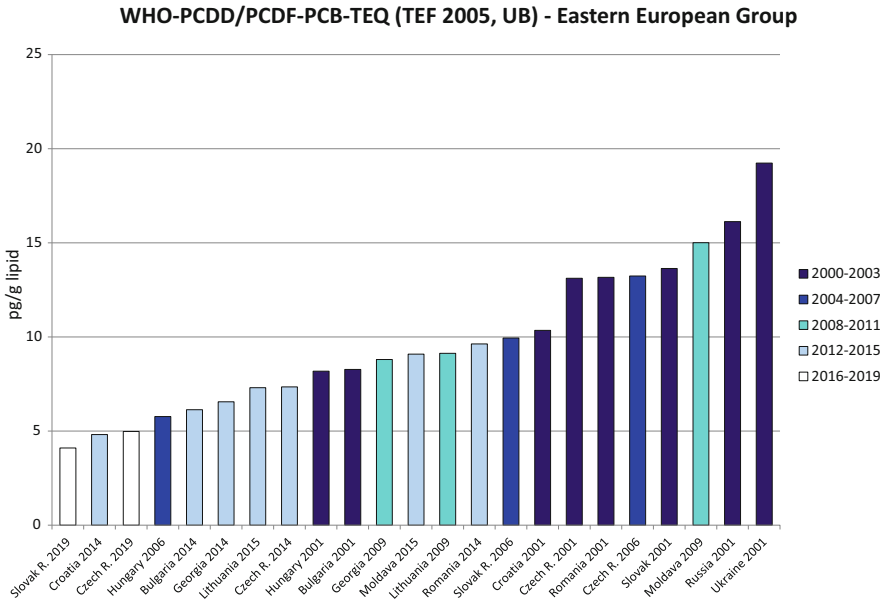

Fig. 20 Results of the 2000–2019 surveys for total TEQ in human milk samples from countries of the Eastern European Group with indication of the period and year of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [TEF 2005, UB])/g lipid)

(2014), Georgia (2014), Lithuania (2014), Moldova (2015), and Romania (2014) were in the range between 6 and 10 pg/g.

Most countries in the third round (2001–2002) submitted two or three pooled samples, however Russia submitted seven samples from various regions of this huge and populous country with concentrations ranging from 13.2 to 24.3 pg/g total TEQ with a median of 16.1 pg/g.

The contribution of PCDD to the total TEQ ranged from 18% for Czech Republic (2001) to 51% for Hungary (2001). The contribution of PCDF ranged from 17% for Russia (2001) to 35% for Bulgaria (2014) and the contribution for dioxin-like PCB was from 27% for Hungary (2001]) to 56% for the Czech Republic (2001) (Fig. 42, in the appendix).

Figure 21 illustrates the range of NDL-PCB concentrations. The highest concentrations were found in 2001 in samples from the Czech Republic (median 502 ng/g $\sum PCB_6$) and the Slovak Republic (median 443 ng/g $\sum PCB_6$). The highest concentration in single pooled sample was 1009 ng/g found in one of the three pooled samples submitted in 2001 from the Czech Republic. In 2006, however, a decrease in levels was apparent in samples from both these countries (Czech Republic: 376 ng/g; Slovak Republic: 255 ng/g). This substantial downward trend continued with samples submitted in 2019 (Czech Republic: 109 ng/g; Slovak Republic: 78 ng/g).

Of interest is the question how non-dioxin-like PCB correlate with TEQ of dioxin-like PCB. Figure 22 illustrates the range of WHO-PCB-TEQ concentrations.

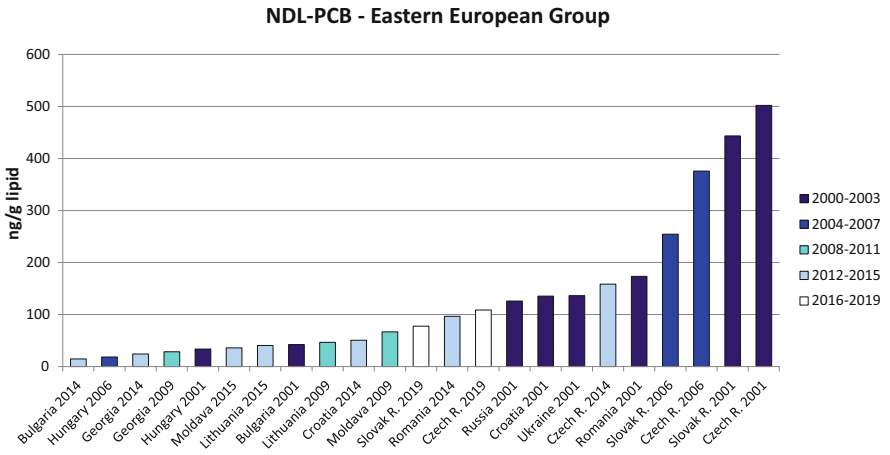

Fig. 21 Results of the 2000–2019 surveys for ∑ PCB6 (ng/g) in human milk samples from countries of the Eastern European Group with indication of the period and year of sample submission

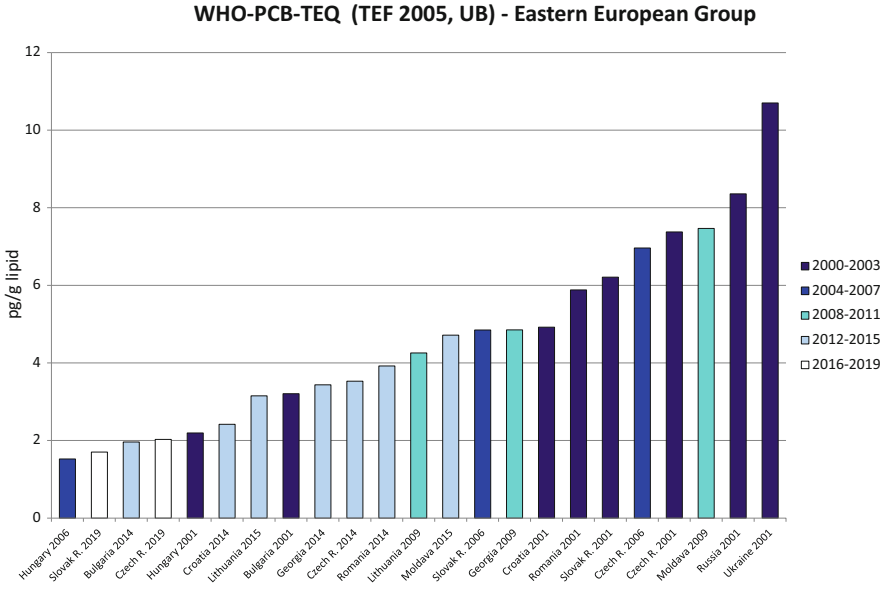

Fig. 22 Results of the 2000–2019 surveys for TEQ from dioxin-like PCB in human milk samples from countries of the Eastern European Group with indication of the period and year of sample submission (pg WHO-PCB-TEQ [TEF 2005, UB])/g lipid)

The highest concentration was found in a single pool sample from Russia (13.6 pg/g). As discussed in Sect. 6, concentrations of TEQ for dioxin-like PCB tend to increase with NDL-PCB concentrations, although with a wide range of variation. As example from the group of Eastern European countries, 13.5 pg/g of TEQ for dioxin-like PCB in the highest contaminated sample from the Czech Republic corresponds to 1009 ng/g $\sum PCB_6$, whereas 13.6 pg/g of TEQ for dioxin-like PCB in a sample from Russia corresponds to 311 ng/g $\sum PCB_6$. Because of this weak correlation between dioxin-like PCB and NDL-PCB in many countries, it can be concluded that the determination of NDL-PCB is no substitute for the determination of dioxin-like PCB.

The Czech Republic has participated in five periods (1992–1993; 200–2003; 2004–2007; 2012–2015; and 2016–2019). The frequent repeated participation over such a long period allows to derive statistically significant temporal trends for this country (Malisch et al. 2023b).

5.5 Western European and Others Group (WEOG)

Figure 23 illustrates the results of total TEQ for the period 2000 and 2019 for countries of the Western European and Others Group. As in the Eastern European Group, the countries from this group submitting samples in the period 2000–2003 also had high concentrations of total TEQ. However, as this UN Regional Group is comprised of industrialized countries from different continents (in addition to

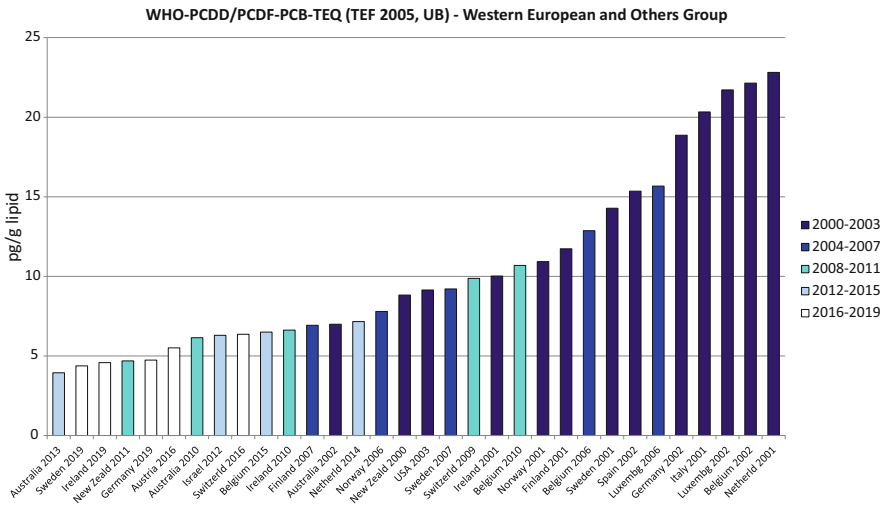

Fig. 23 Results of the 2000–2019 surveys for total TEQ in human milk samples from countries in the Western European and Others Group with indication of the period and year of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [TEF 2005, UB])/g lipid)

Western European countries, Australia, New Zealand, USA, and Israel are included), a differentiation within this group is possible between European and non-European countries. In the 2000–2003 period, the lowest concentrations of total TEQ were found in Australia (2002), New Zealand (2000), and USA (2003) with a range between 7 and 9 pg/g, whereas the Western European countries had concentrations between 10 pg/g (Ireland, 2001) and 23 pg/g (Netherlands, 2001).

Samples collected between 2004 and 2011 were in the range between 4.69 pg/g total TEQ for New Zealand (2011) and 15.7 pg/g for Luxembourg (2006). The trend to lower concentrations continued through 2019 with the lowest concentrations between 4 and 5 pg/g total TEQ found in Australia (2013), Germany (2019), Ireland (2019), and Sweden (2019). Samples from Austria (2016), Belgium (2015), Israel (2012), Netherlands (2014), and Switzerland (2016) were in the range between about 5 and 7 pg/g.

As shown in Fig. 43 (in the appendix), the contribution of PCDD to the total TEQ ranged from 29% for Switzerland (2016) to 61% for Australia (2002). The contribution of PCDF ranged from 11% for New Zealand (2000) to 29% for Ireland (2019) and of dioxin-like PCB from 26% for Australia (2002) to 50% for Switzerland (2016).

Figure 24 illustrates the range of non-dioxin-like PCB concentrations. As in the Eastern European Group, countries of the WEOG submitting samples in the period 2000–2003 also had substantially higher concentrations of $\sum$ PCB_6. As for total TEQ, also for PCB the geographical differences among countries become obvious in this UN region comprising countries from different continents. Starting with the

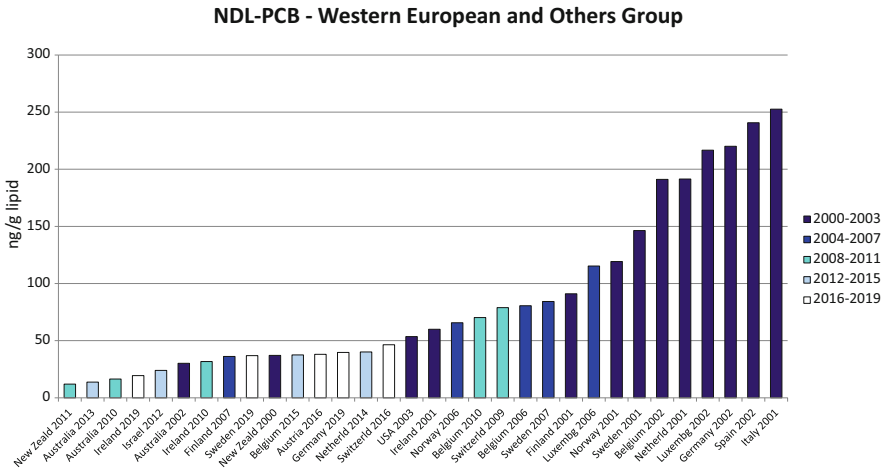

Fig. 24 Results of the 2000–2019 surveys for $\sum$ PCB_6 (ng/g lipid) in human milk samples from countries in the Western European and Others Group with indication of the period and year of sample submission

2000–2003 round, the lowest concentrations were found in Australia (2002), New Zealand (2000), and USA (2003) with a range between 30 and 55 ng/g $\sum PCB_6$, whereas the Western European countries had concentrations between 60 ng/g (Ireland, 2001) and 253 ng/g (Italy, 2001). A substantial decrease in the NDL-PCB concentrations was observed in samples collected between 2008 and 2011. The levels of non-dioxin-like PCB in New Zealand (2011) and Australia (2010) were 12 and 16 ng/g, respectively, whereas the levels in Western European countries ranged between 31 ng/g for Ireland (2010) and 79 ng/g for Switzerland (2009). This difference continued in the 2012–2015 round where Australia (2013) had 14 ng/g, and Israel (2012) had 24 ng/g and the two Western European countries had 38 ng/g (Belgium in 2015) and 40 ng/g (Netherlands in 2014). In comparison to previous rounds, in Germany, Ireland, Sweden, and Switzerland, the lowest concentrations were found in the 2016–2019 round.

Figure 25 illustrates the range of dioxin-like PCB concentrations. The maximum of 11.3 pg WHO-PCB-TEQ/g was found in 2001 in a sample from Italy.

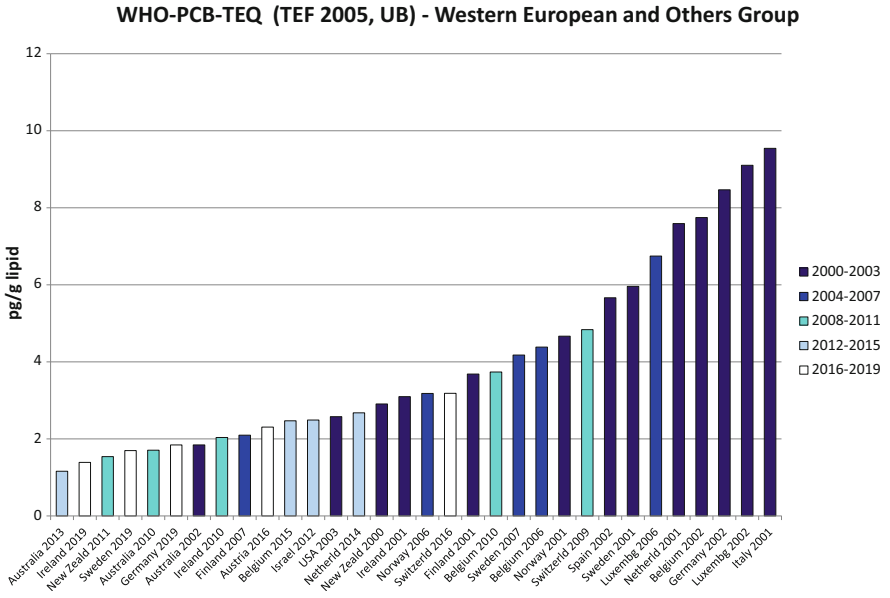

Fig. 25 Results of the 2000–2019 surveys for TEQ of dioxin-like PCB in human milk samples from countries in the Western European and Others Group with indication of the period and year of sample submission (pg WHO-PCB-TEQ [2005 UB]/g lipid)

6 Correlation Between Indicator PCB and Dioxin-like PCB and Between Dioxin-like PCB and PCDF

One question of interest is whether Indicator PCB (non-dioxin-like PCB expressed as ΣPCB_6) can be used to estimate TEQ of dioxin-like PCB (expressed as WHO_{2005}-PCB-TEQ) with PCB 126 being by far the most important congener contributing to toxic equivalency. As shown, European countries have clearly higher PCB concentrations in comparison with countries in the African, Latin American and Caribbean, and Asia-Pacific Groups; furthermore, the greatest variation in PCB concentrations is observed in European countries.

Figure 26 shows this correlation for countries of the Eastern European Group (EEG) and the Western European and Others Group (WEOG). It might be concluded that concentrations of non-dioxin-like PCB concentrations correlate with dioxin-like PCB increase, but with a wide range of variation.

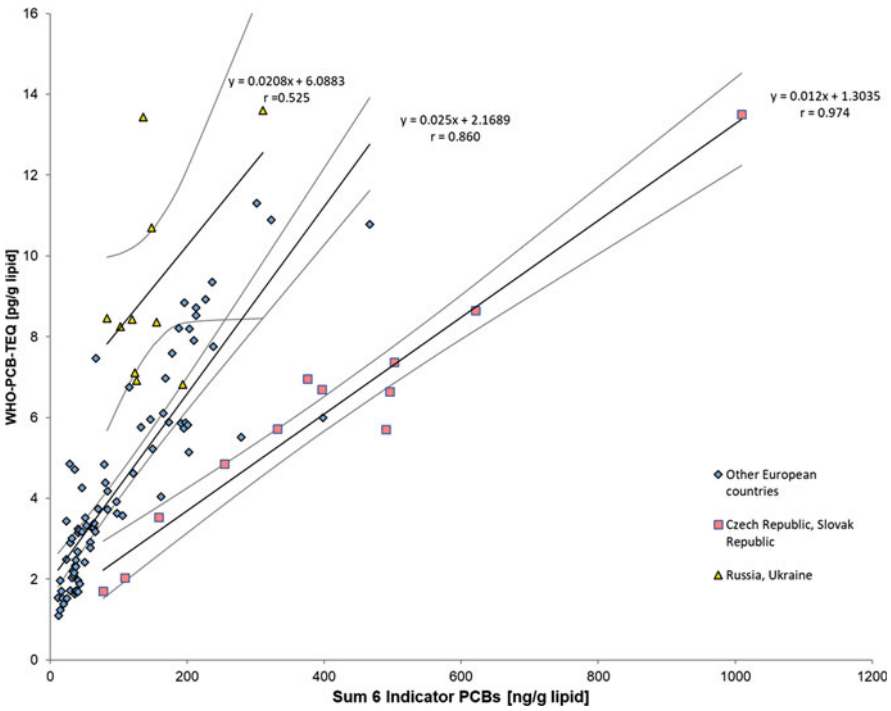

Fig. 26 Correlation between Indicator PCB as sum of the 6 non-dioxin-like PCB (ΣPCB_6) and dioxin-like PCB (WHO_{2005}-PCB-TEQ) in certain countries of the Eastern European Group and Western European and Others Group (linear regression and 95% confidence interval): Includes samples of the Czech Republic (3 in 2001, 1 in 2006, 1 in 2014, and 1 in 2019) and of the Slovak Republic (4 in 2001, 1 in 2006, and 1 in 2019); 10 samples submitted in 2001–2002 from Russia and the Ukraine

Samples from 2001 from the Czech and Slovak Republics had the highest non-dioxin-like PCB concentrations. PCB were produced in Slovakia during the period 1959–1984 in a total amount of about 21,500 tonnes causing elevated human exposure (Kocan et al. 1994, 2001, 2008; Petrik et al. 2001; Drobna et al. 2011). A national study on PCB in blood demonstrated the impact of this former PCB production on PCB blood levels of the population up to 70 km from the production site in the prevailing wind direction (Wimmerová et al. 2015). Assuming that these PCB products were largely used in the former Czechoslovakia and are the main PCB source in these countries, a differentiation is possible between the Czech and Slovak Republics (red squares in Fig. 26) and other European countries (blue diamonds in Fig. 26). Both for samples from the Czech and the Slovak Republics submitted between 2001 and 2019 ($r = 0.974$; $p < 0.001$) and for samples from other European countries ($r = 0.860$; $p < 0.001$), a positive linear correlation was found. However, the slope of the regression line for the samples from the Czech and the Slovak Republics is considerably smaller compared to the other European countries. Therefore, the concentrations of non-dioxin-like PCB in human milk from the Czech and Slovak Republics correlated with lower concentrations of dioxin-like PCB than in other European countries.

In contrast to the Czech and Slovak Republics, most samples collected in 2001–2002 in Russia and the Ukraine (marked as yellow triangles in Fig. 26) lie considerably above the trend line for other European countries. These data points are characterized by comparatively low concentrations of indicator PCB, but relatively high concentrations of dioxin-like PCB ($r = 0.525$; $p > 0.05$).

The congener-specific distributions of major non-dioxin-like and dioxin-like PCB showed a wide range of the TEQ concentrations in numerous commercial PCB formulations from Japan, Germany, USA, Russia, and Poland (Takasuga et al. 2006).

As conclusion, WHO-PCB-TEQ concentrations cannot be estimated exactly from non-dioxin-like PCB concentrations but depend on the correlation between dioxin-like and non-dioxin-like PCB in the various PCB products used in different countries.

Elevated levels of dioxin-like PCB contribute to elevated levels of PCDF as shown in Fig. 27. The correlation between TEQ resulting from dioxin-like PCB and TEQ resulting from PCDF (WHO_{2005}-PCDF-TEQ) for EEG and WEOG countries is observed in that PCDF-related TEQ concentrations increase along with dioxin-like PCB TEQ concentrations (positive correlation with $r = 0.835$, $p < 0.001$) indicating that a considerable share of the WHO-PCDF-TEQ burden stems from PCB. Here, the correlation of samples from Russia, the Ukraine, the Czech Republic, and Slovak Republic is comparable to samples from other European countries; therefore these countries are not separately identified in this figure.

With regard to the contribution of dioxin-like PCB to the total TEQ, attributed mostly by dioxin-like PCB 126, it is relevant to note that EFSA concluded that the current WHO_{2005}-TEF for the PCB 126 might be too high (Sect. 2.4). If this were

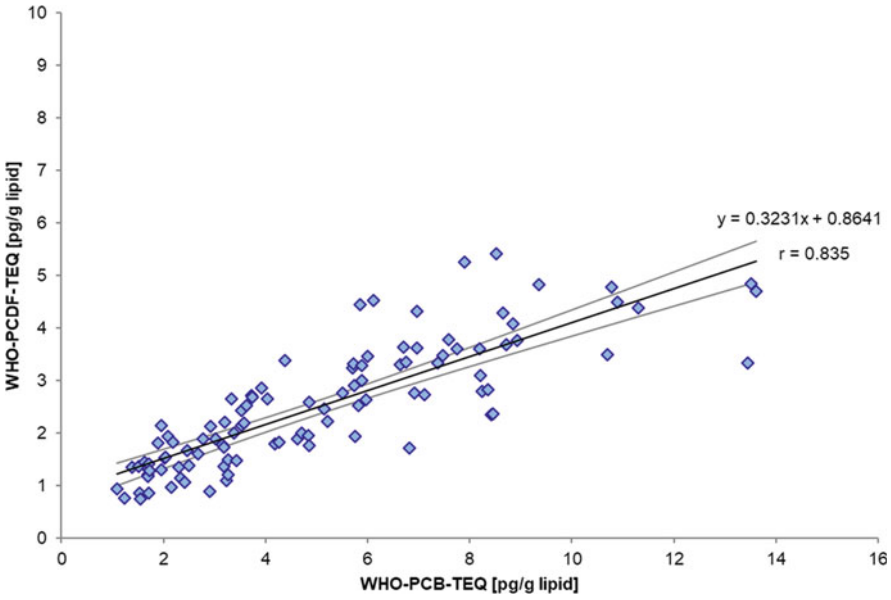

Fig. 27 Correlation between dioxin-like PCB (WHO_{2005}-PCB-TEQ]) and polychlorinated dibenzofurans (WHO_{2005}-PCDF-TEQ) in certain countries of the Eastern European Group and Western European and Others Group (linear regression and 95% confidence interval)

shown to be the case, the contribution of PCB 126 would overestimate the total TEQ and would especially affect European countries. As a result, a general discussion on TEF was proposed by EFSA (2018). Therefore, the TEF for PCB 126 as well as other congeners might be amended in the future.

Another aspect of this re-evaluation is a possible lowering of the Tolerable Weekly Intake (TWI) for the total TEQ for PCB, PCDD, and PCDF. In comparison to the current TWI of 14 pg total WHO_{2005}-TEQ/kg body weight/week (European Commission—Scientific Committee on Food 2001b), the new TWI of 2 pg total WHO_{2005}-TEQ/kg body weight/week proposed by EFSA (2018) is much lower. The requested review by WHO of the WHO_{2005}-TEF values is expected to also give an updated evaluation of the current Provisional Tolerably Monthly Intake (PTMI) of 70 pg total WHO_{2005}-TEQ/kg body weight/month as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO 2001). Lower tolerable intakes of PCDD/PCDF and PCB could raise public health concern as exposures could exceed these intake limits. Possible health risks for the breastfed infant from PCDD/PCDF and PCB as derived from the WHO- and UNEP-coordinated human milk studies are reviewed in Part IV (Van den Berg et al. 2023).

7 Summary

Between 2000 and 2019, the concentrations of specific congeners of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) were determined in a total of 232 pooled human milk samples submitted by 82 countries from all five United Nations regions. These composite samples were analysed over the years in human milk studies that were coordinated by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP). They are considered to be representative of the national average of these analytes in human milk at the time of sampling. Some countries also submitted samples for specific population subgroups or regions.

Results presented here are based on United Nations regions and are not intended for ranking of countries. The highest concentrations of non-dioxin-like PCB were found in 24 European countries (median 118 ng/g (see footnote 1) for the sum of 6 Indicator PCB [PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180; ΣPCB_6], range 14.6–1009 ng/g). Results from 58 countries in other regional groups were generally lower (median 16.4 ng ΣPCB_6/g, range 0.90–96.5 ng/g). Total Toxic Equivalents (TEQ) concentrations of dioxin-like PCB and PCDD/PCDF varied between 1.29 and 49 pg WHO_{2005}-TEQ/g.[1,2] The median of concentrations found in the five UN Regional Groups was highest in countries of the Eastern European Group (12.0 pg WHO_{2005}-TEQ/g) and the Western European and Others Group (10.3 pg WHO_{2005}-TEQ/g). The widest variation in levels of submitted pooled samples was found in countries of the African Group, which ranged from 1.29 to 49 pg WHO_{2005}-TEQ/g. With median concentrations between 4 and 5 pg WHO_{2005}-TEQ/g and maximum levels between 10 and 12 pg WHO_{2005}-TEQ/g, the Pacific region in Asia and the Group of Latin American and Caribbean Countries were at the lower end of the distribution.

One of the objectives of these studies was to generate comparable and consistent monitoring data on the presence of these contaminants in order to identify trends in levels over time. The Guidance Document on the Global Monitoring Plans considers such data on the presence of POPs in the environment and in humans necessary for the evaluation of the effectiveness of the Stockholm Convention. For the sum of the 6 Indicator PCB, the highest country aggregated concentrations by far were found in the period 2000–2003 with a median 123 ng ΣPCB_6/g (range 16.4–502 ng/g) in 26 countries. A considerable downward trend was observed ending with the period 2016–2019 in which the median was 12.7 ng ΣPCB_6/g (range 0.9–109 ng/g) in 43 countries. The total TEQ concentrations for PCB and PCDD/PCDF gradually declined from an initial median of 12.4 pg WHO_{2005}-TEQ/g (range 4.42–23.0 pg WHO_{2005}-TEQ/g) for country aggregated data in the period 2000–2003 to a median

[1] All concentrations are expressed on a lipid basis.

[2] The total TEQ concentrations are expressed in Toxic Equivalents (TEQ) based on Toxic Equivalency Factors recommended by WHO in 2005 and calculated as upper bound values comprising the sum of the TEQ for dioxin-like PCB, PCDD, and PCDF.

of 3.88 pg WHO_{2005}-TEQ/g (range 1.29–11.6 pg WHO_{2005}-TEQ/g) in the period 2016–2019.

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, is greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratory, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the Reference Laboratory was an important aspect for the successful organization of these studies on a global level.

Hae Jung Yoon, Seongsoo Park, and Philippe Verger (Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

The authors thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao, and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

The authors also thank the team at CVUA Freiburg for their performance of the PCDD/PCDF and PCB analyses, in particular Renate Tritschler for her reliable analysis of samples and Helmut Winterhalter for his careful operation of the GC/HRMS systems since the inception of the dioxin laboratory in 1991. Furthermore, the authors thank Indra Peters and Lena Zeug for the analysis and Lukas Jänker, Stefan Leswal, and Jutta Schächtele for GC/HRMS measurements. Nicole Bitomsky, Katharina Djuchin, Annika Maixner, Karin Malisch, Sandra Schill, Biljana Trajkovska, Kerstin Wahl, and Christian Wambold are acknowledged for their scientific support and cooperation with the national coordinators.

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Appendix

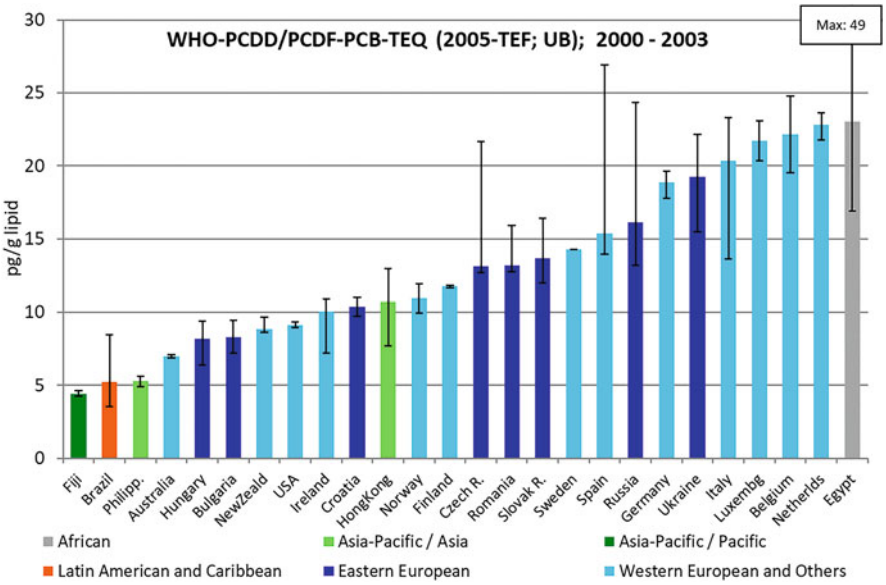

Fig. 28 Country results of the 2000–2003 round for total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) (pg/g lipid; median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

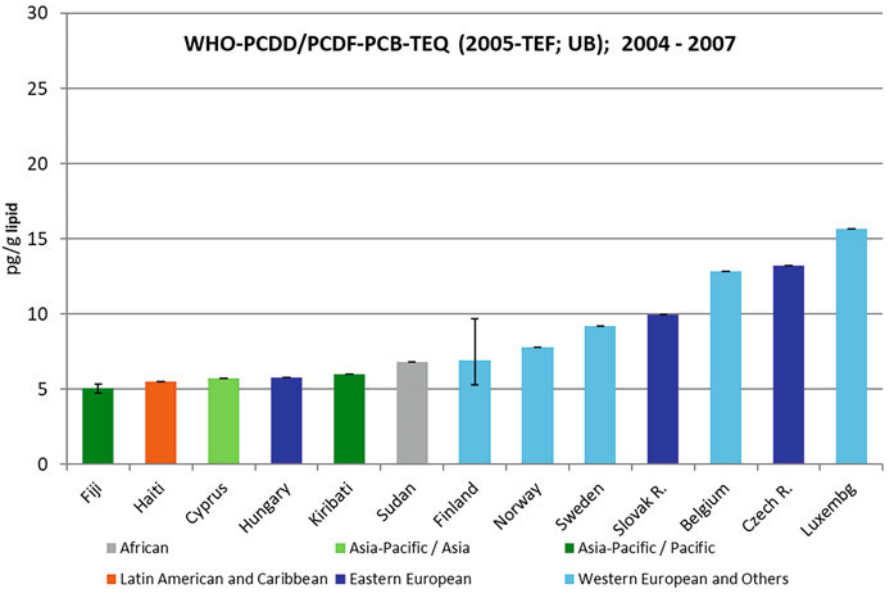

Fig. 29 Country results of the 2004–2007 round for total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) (pg/g lipid; median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

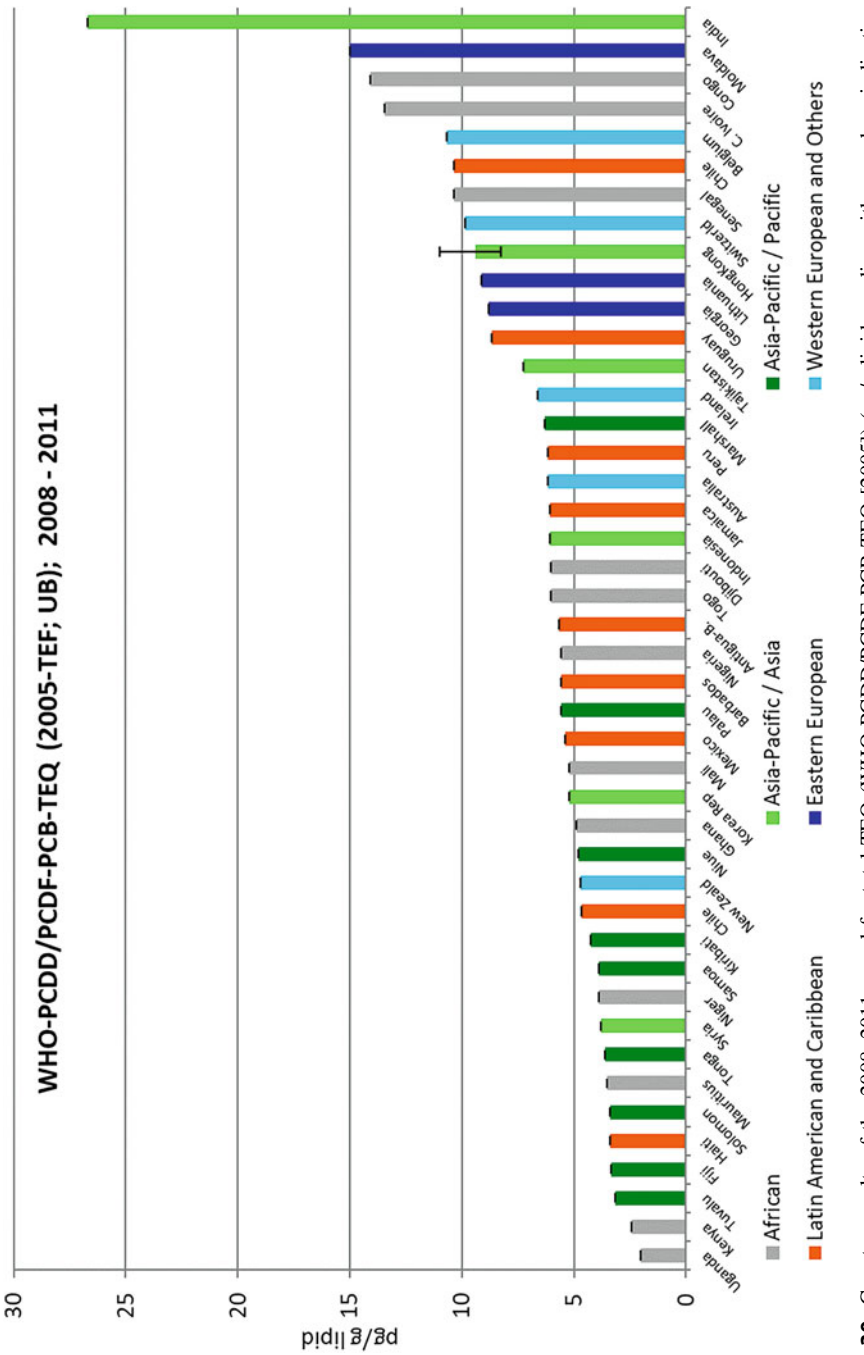

Fig. 30 Country results of the 2008–2011 round for total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) (pg/g lipid; median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

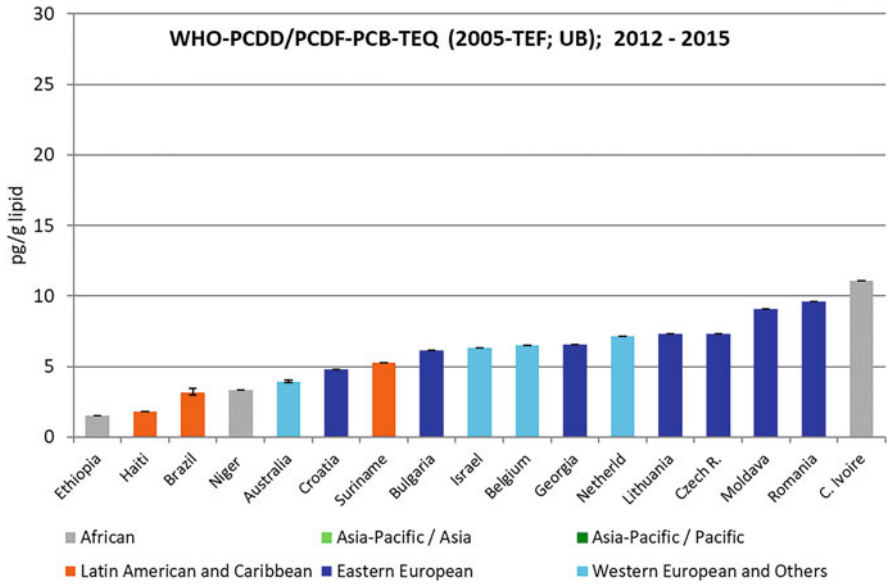

Fig. 31 Country results of the 2012–2015 round for total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) (pg/g lipid; median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

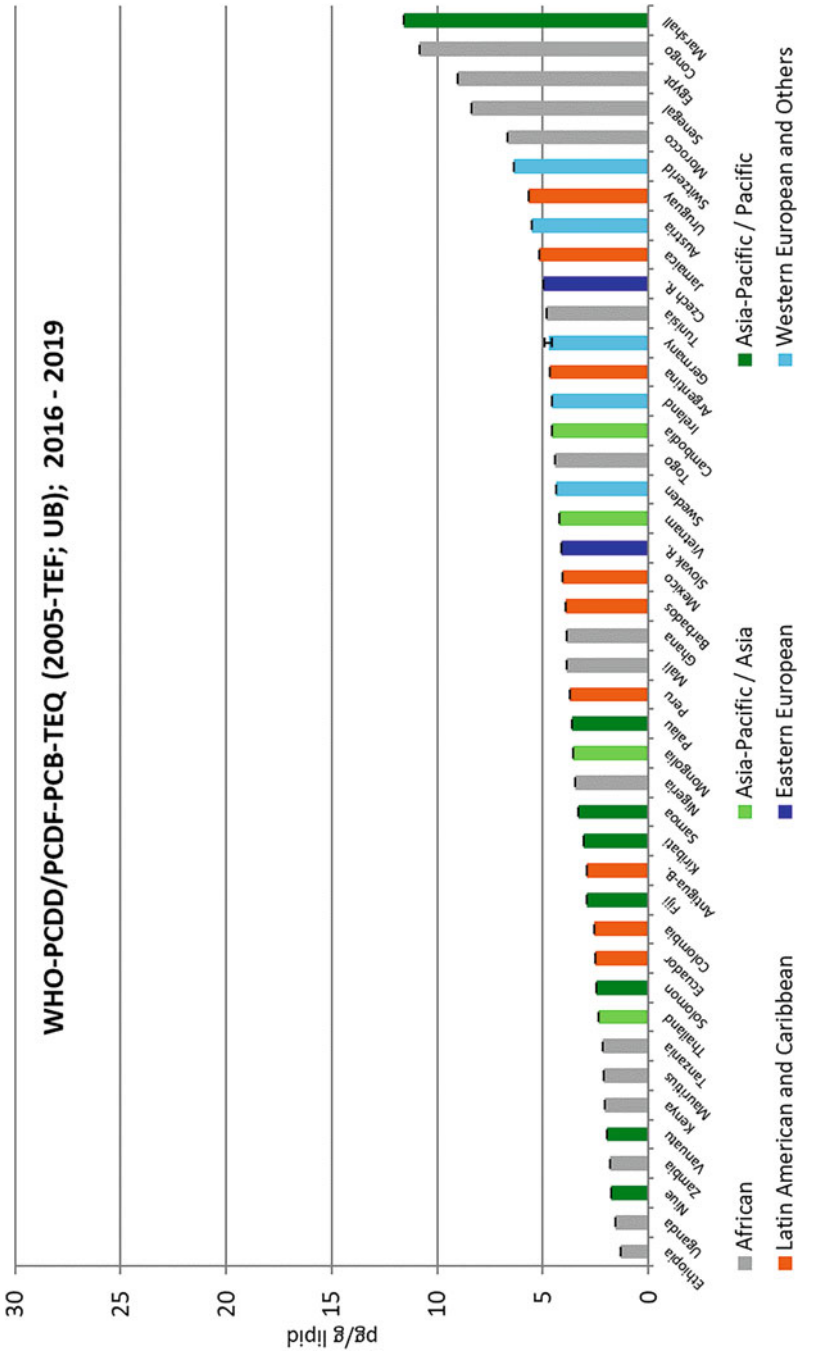

Fig. 32 Country results of the 2016–2019 round for total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) (pg/g lipid; median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

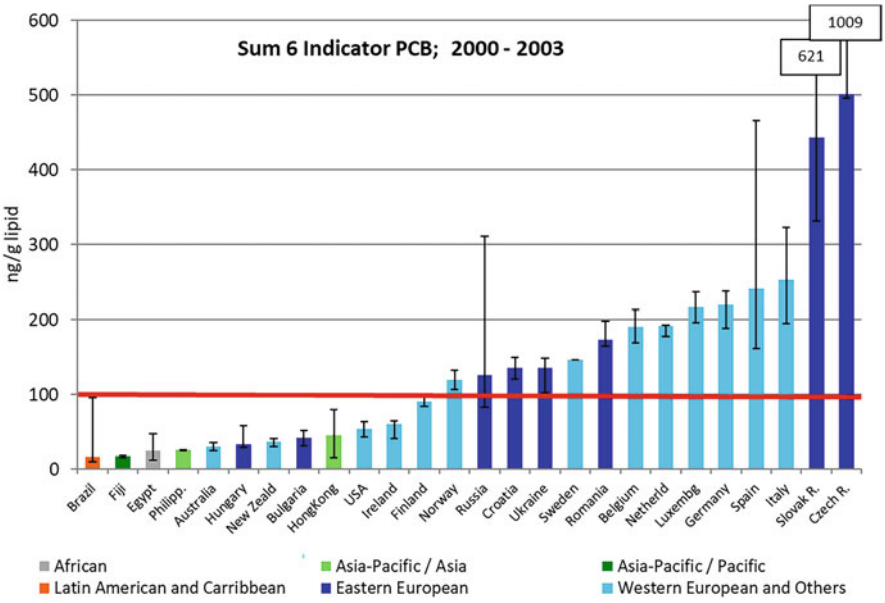

Fig. 33 Country results of the 2000–2003 round for ΣPCB_6 (ng/g lipid) (median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

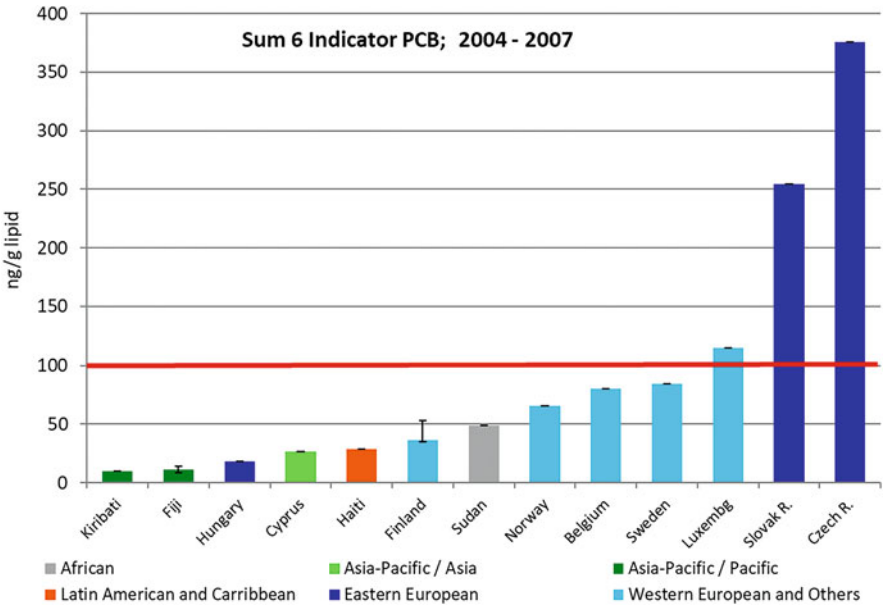

Fig. 34 Country results of the 2004–2007 round for ΣPCB_6 (ng/g lipid) (median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

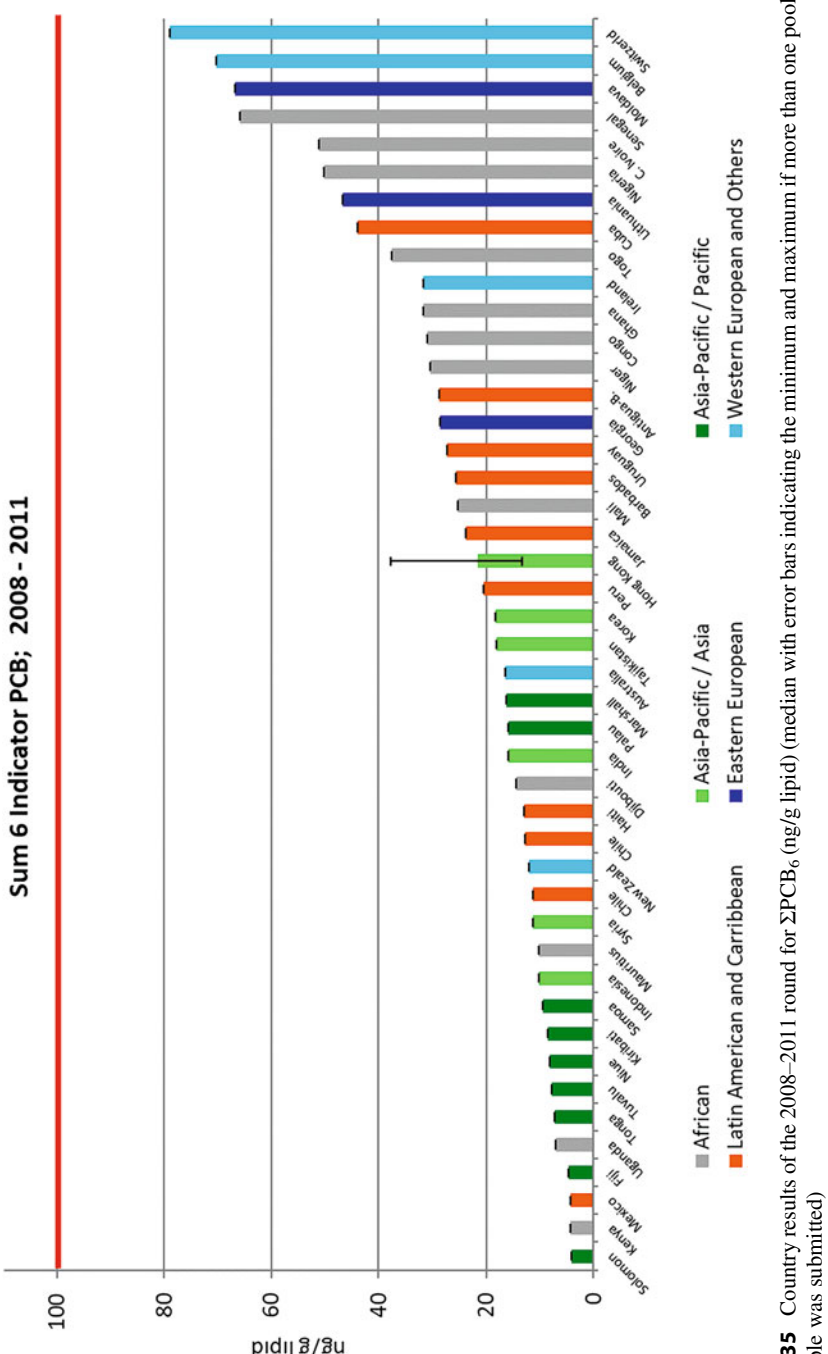

Fig. 35 Country results of the 2008–2011 round for ΣPCB_6 (ng/g lipid) (median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

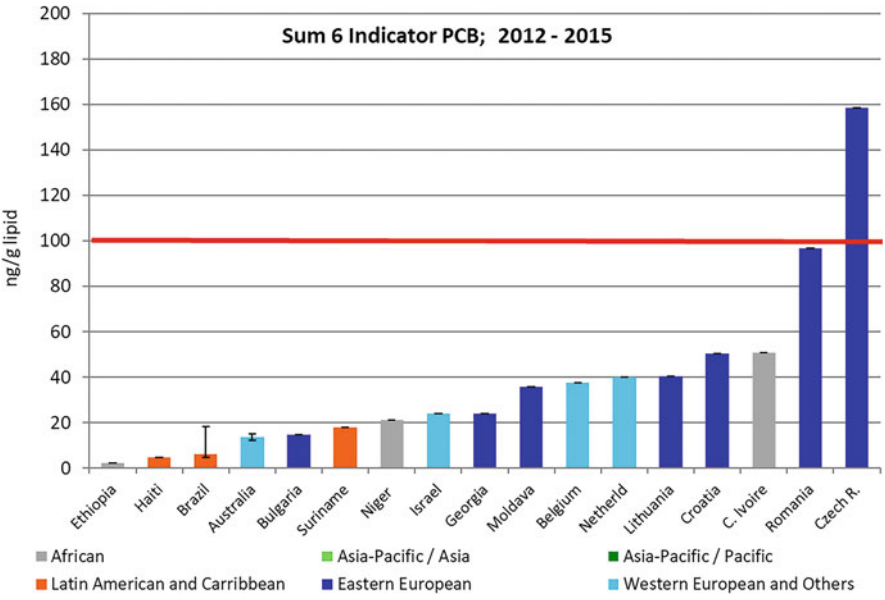

Fig. 36 Country results of the 2012–2015 round for ΣPCB_6 (ng/g lipid) (median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

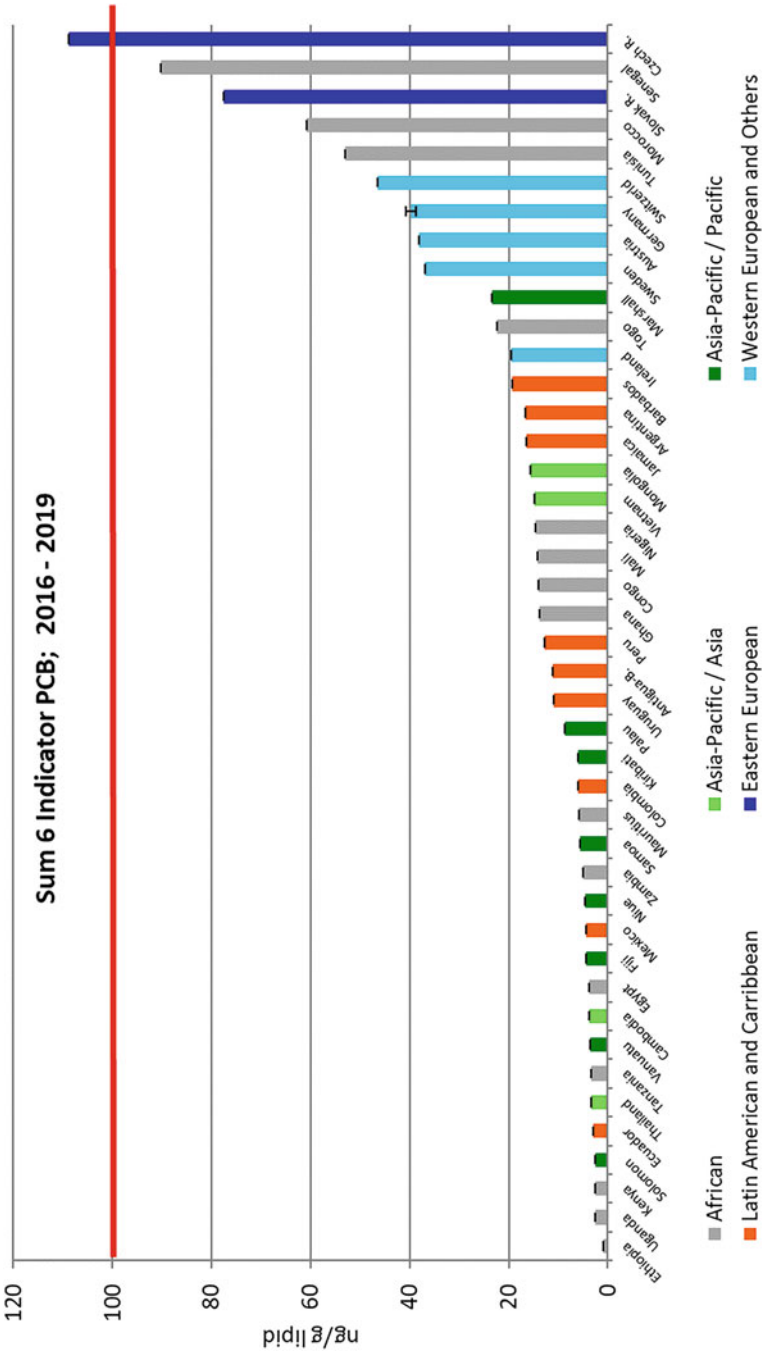

Fig. 37 Country results of the 2016–2019 round for ΣPCB_6 (ng/g lipid) (median with error bars indicating the minimum and maximum if more than one pooled sample was submitted)

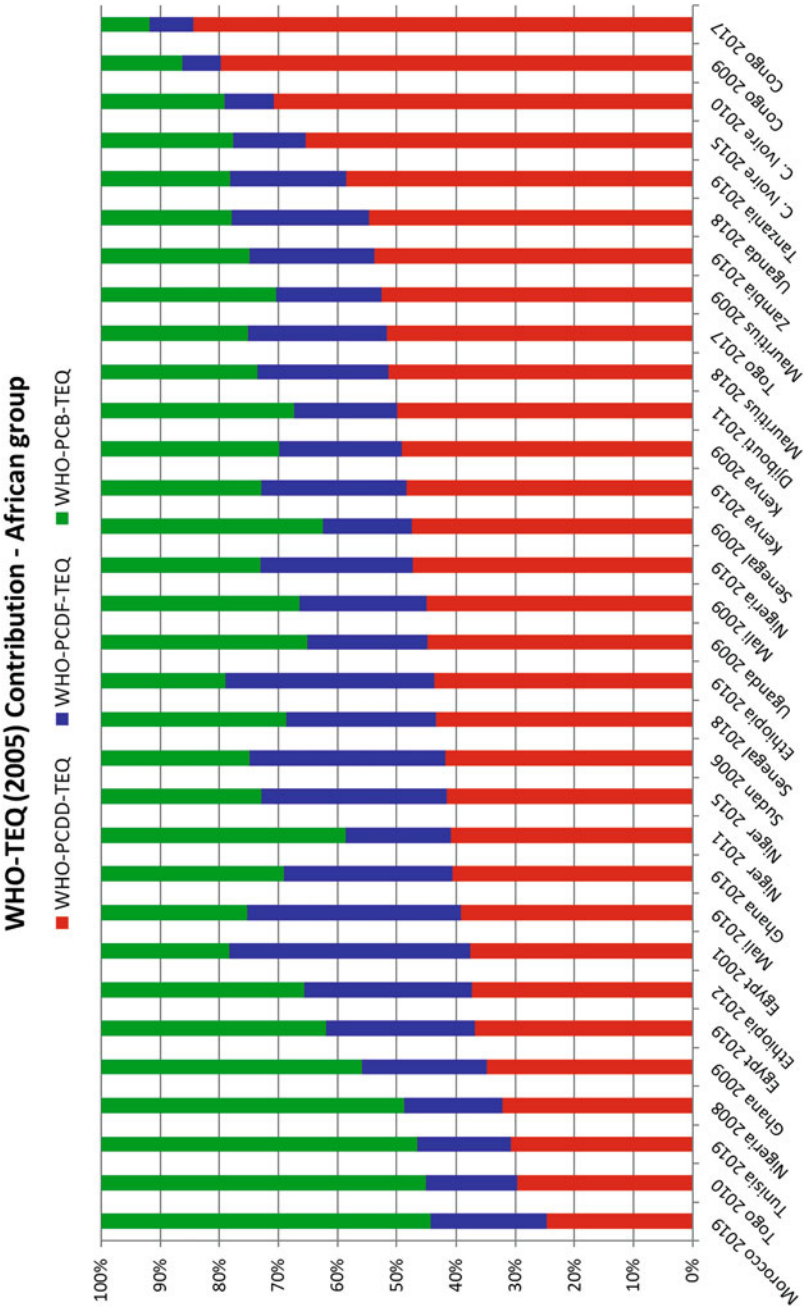

Fig. 38 Relative contribution (%) of toxic equivalents of PCDD (WHO-PCDD-TEQ [2005]), PCDF (WHO-PCDF-TEQ [2005]), and dioxin-like PCB (WHO-PCB-TEQ [2005]) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in human milk samples from countries of the African Group and year of submission

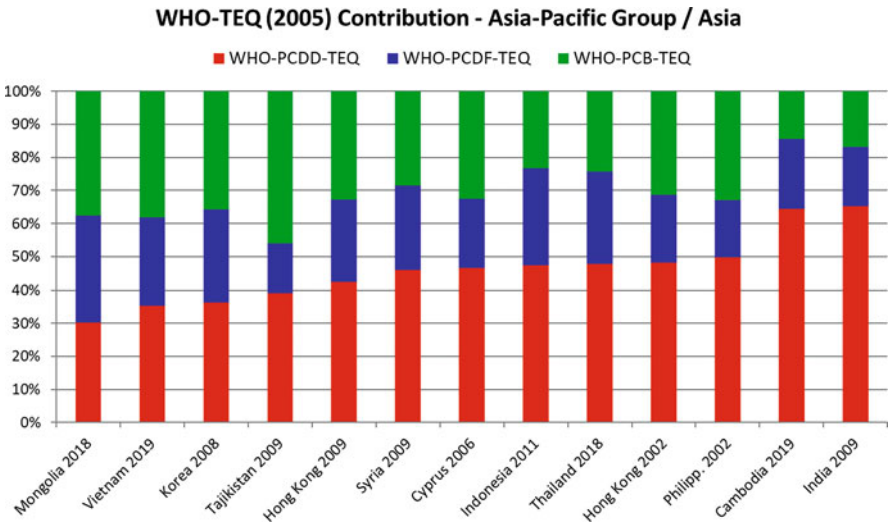

Fig. 39 Relative contribution (%) of toxic equivalents of PCDD (WHO-PCDD-TEQ [2005]), PCDF (WHO-PCDF-TEQ [2005]), and dioxin-like PCB (WHO-PCB-TEQ [2005]) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in human milk from countries from the Asian Subregion of the Asia-Pacific Group and year of submission

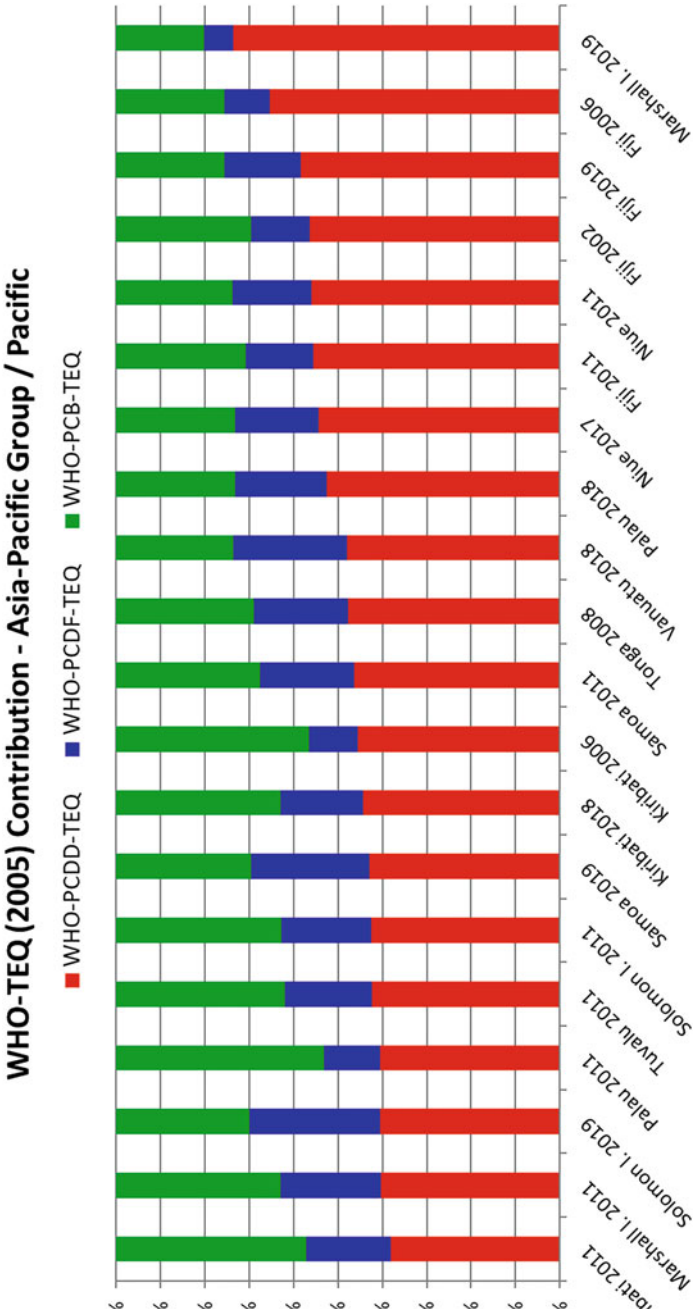

Fig. 40 Relative contribution (%) of toxic equivalents of PCDD (WHO-PCDD-TEQ [2005]), PCDF (WHO-PCDF-TEQ [2005]), and dioxin-like PCB (WHO-PCB-TEQ [2005]) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in human milk from countries from the Pacific Islands Subregion of the Asia-Pacific Group and year of submission

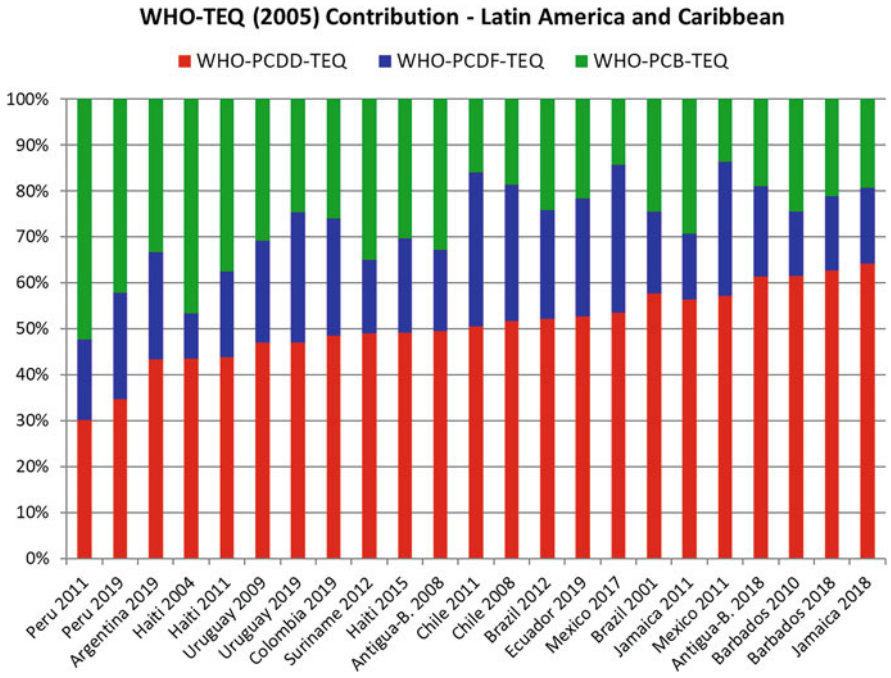

Fig. 41 Relative contribution (%) of toxic equivalents of PCDD (WHO-PCDD-TEQ [2005]), PCDF (WHO-PCDF-TEQ [2005]), and dioxin-like PCB (WHO-PCB-TEQ [2005]) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in human milk from countries from Latin America and the Caribbean and year of submission

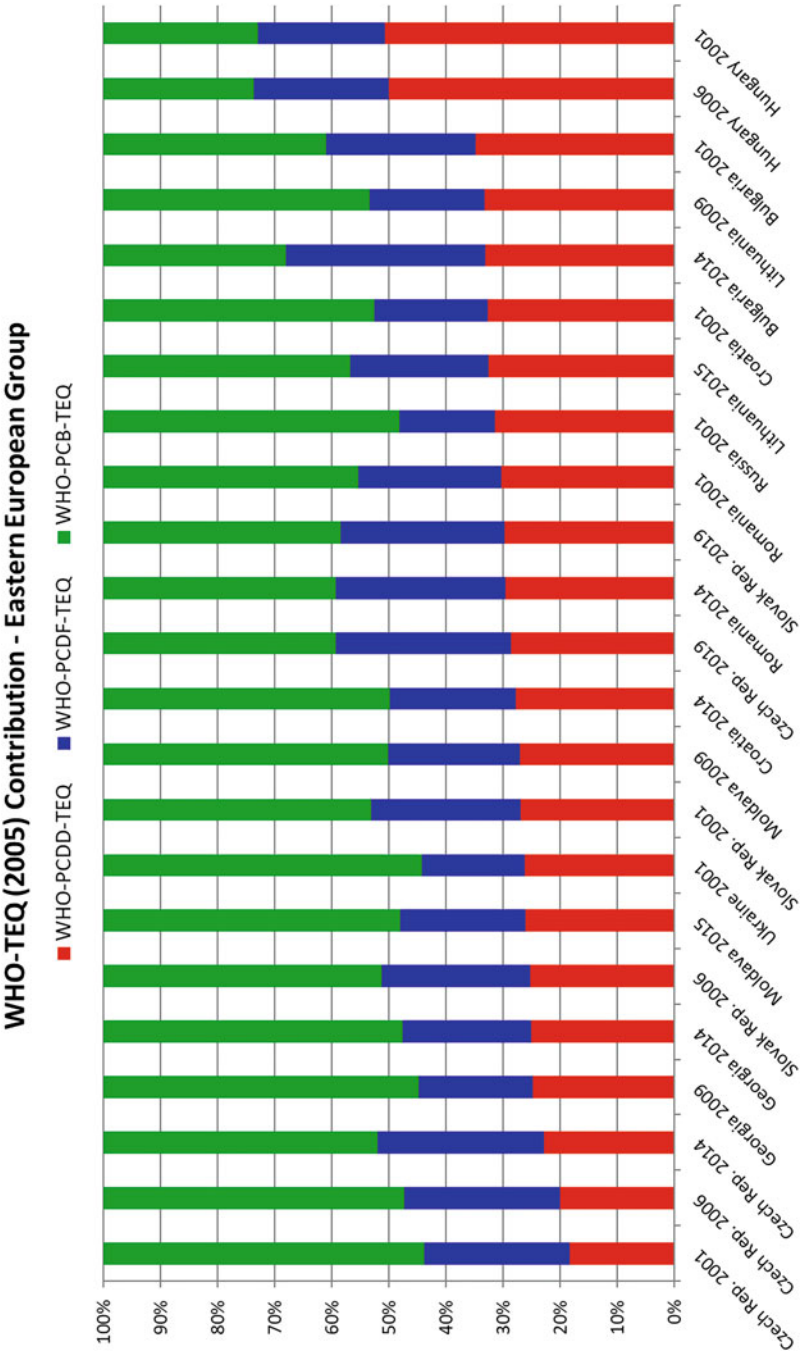

Fig. 42 Relative contribution (%) of toxic equivalents of PCDD (WHO-PCDD-TEQ [2005]), PCDF (WHO-PCDF-TEQ [2005]), and dioxin-like PCB (WHO-PCB-TEQ [2005]) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in human milk from countries of the Eastern European Group and year of submission

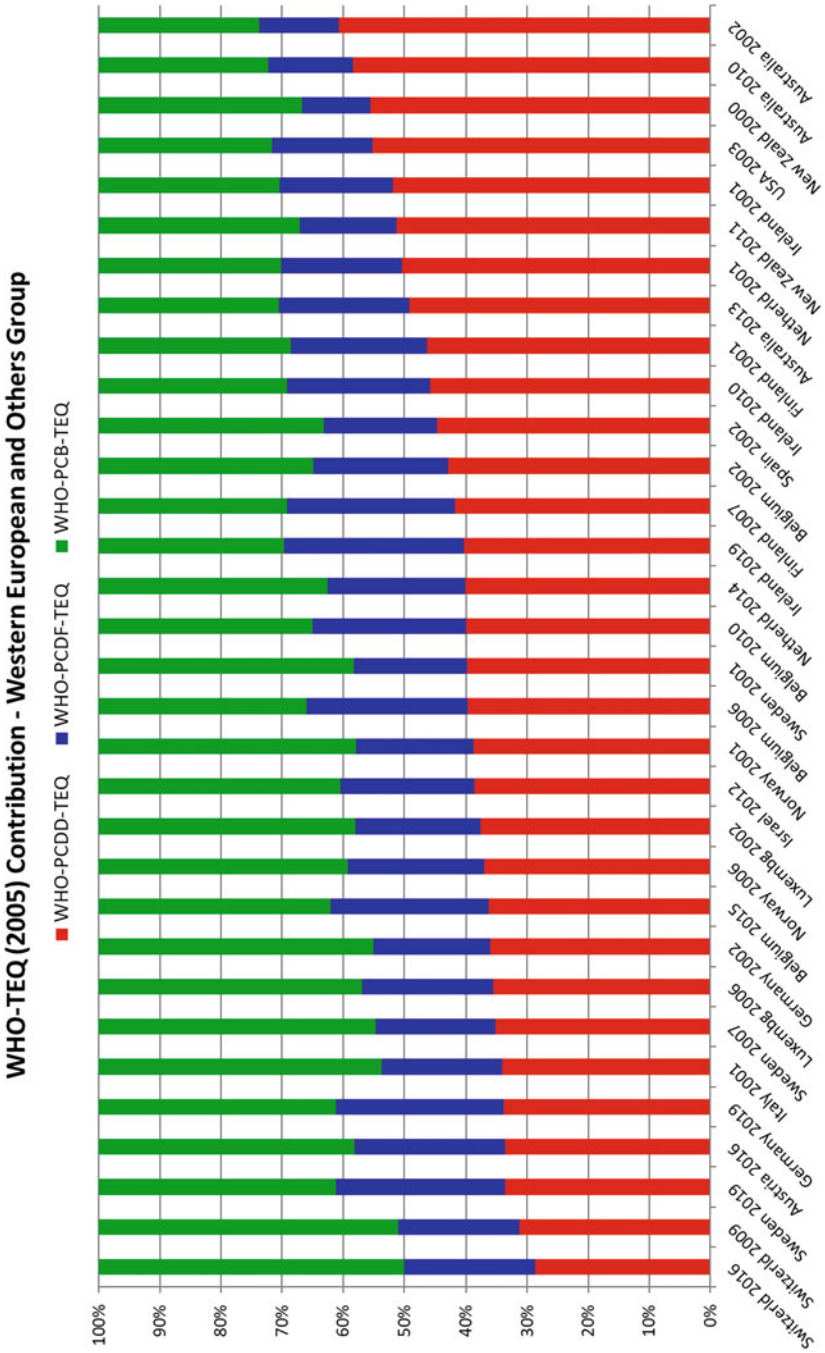

Fig. 43 Relative contribution (%) of toxic equivalents of PCDD (WHO-PCDD-TEQ [2005]), PCDF (WHO-PCDF-TEQ [2005]), and dioxin-like PCB (WHO-PCB-TEQ [2005]) to total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) in human milk from countries from the Western European and Others Group and year of submission

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WHO- and UNEP-Coordinated Exposure Studies 2000–2019: Findings of Organochlorine Pesticides and Industrial Chemicals

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Abstract

The concentrations of a number of organochlorine pesticides and related chemicals and two organochlorine industrial chemicals were determined in 163 pooled human milk samples from 82 countries from all United Nations regions. These countries participated in one or more of the five exposure studies on persistent organic pollutants coordinated by the World Health Organization and the United Nations Environment Programme between 2000 and 2019. The compounds included were aldrin, chlordane, chlordecone, DDT, dicofol, dieldrin, endosulfan, endrin, heptachlor, hexachlorobenzene, hexachlorobutadiene, hexachlorocyclohexanes, mirex, pentachlorobenzene, pentachlorophenol/pentachloroanisole, and toxaphene.

Large differences were found for DDT with the highest concentrations found in Africa. However, the median levels of the DDT concentrations of all samples show a decrease of 72% from the 2000–2003 period to the 2016–2019 period,

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_8

with considerable differences between regions. Due to metabolization of hexachlorocyclohexanes (HCH) in humans, the concentrations of alpha-HCH and gamma-HCH were below the limit of quantification in most human milk samples. The ranges of beta-HCH found in the five periods varied considerably among UN regions, with a maximum found in 2002 in the Asia subgroup of the Asia-Pacific region. A decrease of the median concentrations of all samples of 91% was found from the 2000–2003 period to the 2016–2019 period. In comparison with DDT and beta-HCH, the ranges for hexachlorobenzene (HCB) were much lower with a maximum found in the samples from Eastern Europe. Other organochlorine pesticides and contaminants and their metabolites were found mostly in ranges of low background contamination; some were below the limits of quantification.

Keywords

Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · Legacy and emerging POPs · Organochlorine pesticides · DDT · Hexachlorocyclohexanes (HCH) · Hexachlorobenzene (HCB) · Organochlorine industrial chemicals · Global WHO/UNEP human milk studies · UN regions · Time trends

1 Introduction

A number of organochlorine pesticides used in agricultural or public health applications and industrial chemicals used in various applications are chemically and physiologically stable, and thus persist in the environmental and biological systems. They are also highly lipophilic, which results in their biomagnification in the food chain and bioaccumulation in fatty tissues of animals and humans. For these reasons, they are classified as persistent organic pollutants (POPs) (UNEP 2017).

To protect human health and the environment, the Stockholm Convention on POPs has identified selected POPs to reduce or eliminate their release into the environment (UNEP 2001). The number of POPs initially covered by the convention has expanded considerably since its adoption in 2001 (UNEP 2020). As of 2019, a total of 30 POPs were listed in the Stockholm Convention and subject to Article 16, which requires that they be monitored to evaluate the effectiveness of the convention. The analysis of those POPs in human milk has been recommended as one of the core matrices within the framework of the Global Monitoring Plan on POPs (GMP) with air being the other recommended matrix (UNEP 2019). Of these 30 POPs, 18 are covered in this article.

Human milk surveys are reviewed in this compendium from various aspects. As background information for this article, the general introduction (Part I) gives a review of human milk surveys on POPs from a historical perspective (Fürst 2023), an overview of the World Health Organization (WHO)- and United Nations Environment Programme (UNEP)-coordinated exposure studies performed between

1987 and 2019 (Malisch et al. 2023a), and a review on the Stockholm Convention on POPs and its implementation summarized by regional and global monitoring reports (Šebková 2023). The analytical methods used for determination of chlorinated pesticides and industrial chemicals in samples of the WHO/UNEP-coordinated exposure studies and their validation are presented in Part II (Hardebusch et al. 2023).

In this paper, the concentrations of the pesticides aldrin, chlordane (comprising cis-, trans- and oxychlordane and nonachlor), chlordecone, dicofol, dichlorodiphenyltrichloroethane (DDT, comprising the *p*,*p*′- and *o*,*p*′-isomers of DDT and its metabolites DDE and DDD), dieldrin, endosulfan (comprising alpha- and beta-endosulfan and endosulfan sulfate), endrin (including endrin ketone), heptachlor (including heptachlor epoxide), hexachlorobenzene (HCB), gamma-hexachlorocyclohexane (γ-HCH, lindane) and by-products alpha-HCH and beta-HCH, mirex, pentachlorophenol and its metabolite pentachloroanisole and toxaphene and of the industrial chemicals pentachlorobenzene and hexachlorobutadiene are reported for the five WHO/UNEP exposure studies between 2000 and 2019.

Results of the WHO/UNEP-coordinated exposure studies for the 2000–2008 period (Malisch et al. 2008) and the 2008–2009 round (Malisch et al. 2010) showed that in comparison with DDT, the levels of other chlorinated pesticides were low. Human milk data of these studies in the period 2008–2010 demonstrated the worldwide presence of POPs in human tissues in 32 less developed countries (Fiedler et al. 2013). A comprehensive report for the 6th Conference of the Parties to the Stockholm Convention in 2013 provided an overview on all samples of the three studies spanning the period 2000–2012. It revealed large global differences among various POPs (UNEP 2013). Also, aspects of a risk-benefit assessment of breastfeeding were addressed that were published later in more detail (van den Berg et al. 2016).

Worldwide trends in DDT concentrations in human breast milk were assessed compiling data since 1951 until the end of the 1990s (Smith 1999). A global overview on the spatial and temporal trends of Stockholm Convention POPs in breast milk reviews scientific publications between 1995 and 2011 (Fång et al. 2015). The regional and global monitoring reports for the GMP assess datasets in the core media—ambient air, human tissues (human breast milk or blood), and water for hydrophilic POPs, but also other media such as soil, biota, plants are used to support interpretation of observed levels and their trends (UNEP 2022a).

All substance-specific data of the WHO/UNEP-coordinated exposure studies are deposited at the Global Monitoring Plan Data Warehouse (GMP DWH), which can be publicly accessed (Global Monitoring Plan Data Warehouse 2020).

A total of 82 countries participated in one or more of the five studies conducted between 2000 and 2019 and submitted pooled human milk samples, which are considered to represent a country or, in some cases, a subgroup of a country. The results for 163 pooled samples are discussed from various perspectives in the following sections, namely: Sect. 2 General aspects; then Results and Discussions in Sect. 3.1 for DDT; in Sect. 4 for hexachlorocyclohexanes (alpha-HCH, beta-HCH, and gamma-HCH); in Sect. 5 for HCB; in Sect. 6 for other organochlorine pesticides;

and, in Sect. 7 for organochlorine industrial chemicals. Section 8 summarizes the findings.

2 General Aspects

2.1 Countries, UN Regions, Protocol, and Analytes Selected

An overview of the scope, protocols for collection of samples and participation of countries with classification in UN regions and temporal differentiation is given in the general introduction in Part I (Malisch et al. 2023a). In brief, the collection of a number of individual samples and preparation of representative pooled samples in all rounds were supervised by a national coordinator in each country following the WHO/UNEP-standardized protocols. Equal aliquots of individual samples were combined to give a composite sample, which was considered to represent the average levels of POPs for a country at the time of sampling. The pooled samples were sent to WHO/UNEP Reference Laboratories for analysis.

WHO was initially focused on polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) and had conducted two studies prior to 2000 (WHO 1989; WHO 1996). A third study was performed from 2000 to 2003 with participation of 26 countries/areas. With the adoption of the Stockholm Convention on Persistent Organic Pollutants in 2001 and in anticipation of its ratification in 2004, a pilot study was conducted in 2003 by WHO and the WHO Reference Laboratory for POPs located at the State Institute for Chemical and Veterinary Analysis of Food (CVUA) in Freiburg, Germany. The study aimed to assess the feasibility of measuring in human milk the initial twelve POPs listed by the convention. In addition, 9 more POPs were included in the study, which were later added to the convention, resulting in the analysis of a total of 21 chemicals. For the pilot study, the fat component extracted from human milk samples from the third round (2000–2003) was used, if sufficient sample amount was retained, which was the case for 16 countries. The results confirmed the advantages of using human milk as a matrix to monitor these POPs.

Similarly, when the 2016 round started, a total of 23 chemicals were required to be analyzed but an additional 7 POPs were about to be added to the convention. Therefore, the Reference Laboratory for chlorinated and brominated substances at CVUA Freiburg, Germany, and for perfluorinated compounds at Örebro University, Sweden, analyzed the seven additional POPs in order to have data on the complete picture of the then listed 30 chemicals for samples of the 2016–2019 period as baseline data for future effectiveness evaluations. Of the total of 30 chemicals as listed by the convention up until 2019, 18 are covered in this article, including hexachlorobutadiene, pentachlorophenol and dicofol from the 7 newly added POPs (for expansion of the analytes of interest over time by the Stockholm Convention and the Reference Laboratories, see Malisch et al. 2023a).

In accordance with the implementation of the GMP, parties report flexibly through one of the five United Nations regional organization groups. Therefore,

countries participating in UNEP projects for the GMP are classified according to one of these five UN geopolitical groups: (1) African Group, (2) Asia-Pacific Group, (3) Eastern European Group, (4) Group of Latin American and Caribbean Countries (GRULAC), and (5) Western European and Others Group (WEOG). Note that Australia, Israel, New Zealand, and the USA (being informally a member) are included as "Others" in WEOG countries, whereas Cyprus belongs to the Asia-Pacific Group (for participating countries and regional distribution, see Section 6 in Malisch et al. 2023a). It should, therefore, be noted that these results are not intended to be used for the ranking of individual countries.

2.2 Number of Samples and Aggregation of Data

A total of 82 countries submitted pooled samples during one or more of the five studies conducted from 2000 to 2019. Due to the particular scope at beginning of a study with regard to the expansion of analytes of interest over time, 163 pooled samples were analyzed for various organochlorine pesticides and industrial chemicals.

In the 2000–2003 study, countries were encouraged to submit at least two pooled samples, whereas in the following rounds, usually one pooled sample was submitted by a country. If a country had sent two or more pooled samples in a certain round, the **median of the individual results** was used for **aggregation** purposes. This yielded 14 median results that are used to represent the country for that round, namely from Hong Kong in 2002 and 2009; Philippines in 2002; Brazil in 2002 and 2012; Fiji in 2002 and 2006; Australia in 2013; Finland in 2007; Germany in 2002 and 2019; Luxembourg in 2002; Spain in 2002; and the USA in 2003. In contrast, a total of 120 results were obtained from the **single pooled sample** submitted by countries in a certain round. Thus, 134 **country results** are available for the "one country—one result for a certain period" approach. The detailed data for all 163 pooled samples is contained at the POPs Global Monitoring Plan Data Warehouse and can be publicly retrieved (Global Monitoring Plan Data Warehouse 2020).

2.3 Analysis and Complexes of DDT, Chlordane, Heptachlor, Endrin, and Endosulfan

The analytical methods for determination of organochlorine pesticides and industrial chemical complexes and their validation are presented in Part II (Hardebusch et al. 2023). All concentrations are reported on lipid weight basis.

For many chemicals, it is recommended to determine not only concentrations of the parent molecule but also to include certain metabolites, degradation products, and/or by-products during manufacture. For this reason, the Stockholm Convention provides guidance on the analytes to be included under each parent POP (UNEP 2019; Malisch et al. 2023a). The sum of the parent POP and its other compounds of concern can be calculated based on the determined levels with two options:

(1) without correction for molecular weight (mass basis), (2) after correction for molecular weight (molar basis).

The EU regulation on maximum residue levels in feed and food defines the residues for DDT, chlordane, heptachlor, and endosulfan with the addition of "expressed as …", e.g., "DDT (sum of *p,p′*-DDT, *o,p′*-DDT, *p-p′*-DDE and *p, p′*-TDE (DDD) expressed as DDT)" (Regulation (EC) No 396/2005). Therefore, according to the "Pesticide Guidelines," the sum of the components is calculated by adjusting for different molecular weights ("correction factors" or "conversion factors") (DG-SANTE 2021) This regulatory approach is harmonized internationally with that of the OECD (OECD 2011).

The sum parameters in this article referred to as "complexes" were calculated following the principles of the EU regulation by use of the correction factors as listed in Table 1 and applying the "lower bound approach," which uses only quantifiable results (Malisch et al. 2008).

2.4 Background Concentrations

Background concentrations are defined as that portion of the measured human milk levels that is found in the absence of specific sources and therefore is not attributable to a known exposure, e.g., to use of the chemical of interest or to emissions within the study area. In contrast to findings of high concentrations, e.g., after use of chemicals, after a sufficient long withdrawal period for many POPs the levels are described as "low background levels." However, the term "background level" does not imply per se any level of safety. With respect to potential adverse effects, risk assessments need to consider many factors, including the toxicity of the chemical of interest and the found concentration range. For human milk, potential adverse effects have to be balanced against positive health aspects for (breastfed) infants (van den Berg et al. 2016).

3 Results and Discussion

3.1 DDT

Dichlorodiphenyltrichloroethane (DDT) belongs to the group of the "12 Initial POPs" listed in Annex B (Restriction) to the Stockholm Convention (UNEP 2020). UNEP's DDT web section covers various aspects on DDT (e.g., overview; COP decisions; technical assistance to Parties; guidance; DDT register; DDT toolkit) and information on the Global Alliance for promoting a global partnership on the development and deployment of alternative products, methods, and strategies to DDT for disease vector control (UNEP 2022b).

Commercial DDT is a mixture mainly of the desired *para-para′ substituted* isomer (*p,p′*-DDT = 4,4′-DDT) as major component and the *ortho-para′ substituted* isomeric impurity (*o,p′*-DDT = 2,4′-DDT). Due to degradation

Table 1 Complexes of DDT, chlordane, heptachlor, endrin, and endosulfan and correction factors for molecular weight used for the calculation of the sum parameters

	Correction factors
DDT	
op′-DDT	1
pp′-DDT	1
op′-DDD	1.108
pp′-DDD	1.108
op′-DDE	1.115
pp′-DDE	1.115
DDT complex[a]	
Chlordane	
cis-chlordane (alpha-chlordane)	1
trans-chlordane (gamma-chlordane)	1
Oxychlordane	0.967
cis-nonachlor	0.923
trans-nonachlor	0.923
Chlordane complex *(cis+trans+oxy)*[b]	
Chlordane group *(all 5 analytes)*[c]	
Heptachlor	
Heptachlor	1
cis-heptachlor epoxide	0.959
trans-heptachlor epoxide	0.959
Heptachlor complex[d]	
Endrin	
Endrin	1
Endrin ketone	1
Endrin complex[e]	
Endosulfan	
Alpha-endosulfan	1
Beta-endosulfan	1
Endosulfan sulfate	0.962
Endosulfan complex[f]	

[a]Sum of all detected analytes, calculated as DDT
[b]Residue definition according to GMP Guidance, 2007 (UNEP 2007) and in food legislation: sum of cis- and trans-chlordane and oxychlordane, calculated as chlordane *(without nonachlor)*
[c]According to GMP Guidance (UNEP 2019): sum of all 5 recommended analytes *(including nonachlor)*
[d]Sum of all detected analytes, calculated as heptachlor
[e]Sum of all detected analytes, calculated as endrin
[f]Sum of all detected analytes, calculated as endosulfan

and metabolization, in humans the transformation products 4,4′-DDE (dichlorodiphenyldichloroethylene) and 2,4-DDE, respectively, and 4,4′-DDD (dichlorodiphenyldichloroethane) and 2,4′-DDD, respectively, are of interest (UNEP 2007; Fürst 2023).

For calculation of the summarizing parameter "DDT complex," correction factors for molecular weight as shown in Table 1 were applied (see Sect. 2.3). The detailed results for DDT complex (including the isomers of parent molecules and metabolites) are publicly available (Global Monitoring Plan Data Warehouse 2020). As a general conclusion, in most human milk samples concentrations of *o,p′*-DDD, *p,p′*-DDD and *o,p′*-DDE were below the limit of quantification (0.5 µg/kg lipid) and their contribution to DDT complex was below 1%. In most human milk samples, *p,p′*-DDE contributes about 95% while *p,p′*-DDT contributes about 5% and *o,p′*-DDT 0.5%. However, in cases of more recent use or contamination, considerably higher contributions of *p,p′*-DDT (maximum found: 46%) and slightly increased concentrations of *o,p′*-DDT (maximum found: 4.2%) can be found.

3.1.1 General Comparison of Ranges

A suitable starting point for the discussion is the comparison of the ranges among UN regions found in the five studies performed over 20 years. Large differences covering three orders of magnitude were found, with a minimum of 17 µg DDT complex/kg lipid found in 2019 in a country from Africa and a maximum of 23,500 µg DDT complex/kg lipid found in 2012 in another country from Africa. The median of 134 country results (see Sect. 2.2) was 255 µg DDT complex/kg lipid.

As the five studies had slightly different time lengths, the comparison of results for the regions is presented in five equal periods of 4 years each, namely: 2000–2003, 2004–2007, 2008–2011, 2012–2015, and 2016–2019 (Malisch et al. 2023a). Table 2 compiles the most important statistical data (number of samples, minimum, median, mean, and maximum) for the 134 country results for the five UN regions (here with split of the of the Asia-Pacific Group into two subgroups, namely the Asian and the Pacific Island countries).

As **general estimation of time trends**, the *median* of the DDT complex concentrations of all country results shows a decrease of 72% from the 2000–2003 period (median for 16 countries: 445 µg/kg lipid) to the 2016–2019 period (median for 43 countries: 125 µg/kg lipid). The downward trend between these two end periods was found in all regions, but with considerable differences among regions and a great variation among the three rounds in the middle, as illustrated by Fig. 1. In all groups, the median of the DDT complex concentrations was higher in the 2000–2003 period than in the 2016–2019 period. However, in the three rounds in-between, a considerable variation within UN regions was observed; obviously with a substantial maximum in Latin American and Caribbean countries in the 2004–2007 period and elevated levels also in Africa at that time period. Therefore, conclusions on time trends in the different groups cannot easily be drawn.

If calculated as the *mean*, the DDT complex concentration was higher in the 2000–2003 period than in the 2016–2019 period in all regions except Africa. Again, in the three rounds in-between, a considerable variation was observed, with a substantial maximum in Africa in the 2012–2015 period (Fig. 2).

As a result, the median, mean, and ranges of DDT complex concentrations found in the UN regions in five periods over these 20 years cannot be used directly to derive continuous time trends. A closer look into the details of Table 2 reveals the

Table 2 Minimum, median, mean, and maximum concentrations of DDT in human milk (expressed as μg DDT complex/kg lipid) in the five UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands) for the five periods between 2000 and 2019 based on 134 country results (see Sect. 2.2) with the number of country results [*N*] in the respective period

Period	*N*	Minimum	Median	Mean	Maximum
		African	**African**	**African**	**African**
2000–2003	1	396	396	396	396
2004–2007	1	1300	1300	1300	1300
2008–2011	12	233	985	1060	1930
2012–2015	3	297	491	8090	23,500
2016–2019	15	17	195	724	7100
		Asia-Pacific/ Asia	**Asia-Pacific/ Asia**	**Asia-Pacific/ Asia**	**Asia-Pacific/ Asia**
2000–2003	2	1250	1410	1410	1580
2004–2007	1	324	324	324	324
2008–2011	6	136	691	2200	8490
2012–2015	0				
2016–2019	4	45	130	194	473
		Asia-Pacific/ Pacific Islands	**Asia-Pacific/ Pacific Islands**	**Asia-Pacific/ Pacific Islands**	**Asia-Pacific/ Pacific Islands**
2000–2003	1	1340	1340	1340	1340
2004–2007	2	189	439	439	689
2008–2011	9	56	275	784	4760
2012–2015	0				
2016–2019	8	31	115	265	1390
		Latin American and Caribbean	**Latin American and Caribbean**	**Latin American and Caribbean**	**Latin American and Caribbean**
2000–2003	1	428	428	428	428
2004–2007	1	2830	2830	2830	2830
2008–2011	10	132	200	297	695
2012–2015	3	263	502	719	1390
2016–2019	9	46	103	195	622
		Eastern European	**Eastern European**	**Eastern European**	**Eastern European**
2000–2003	4	461	610	716	1180
2004–2007	3	361	377	396	449
2008–2011	3	282	632	911	1820
2012–2015	7	64	304	576	1490
2016–2019	2	192	219	219	246
		Western European and Others	**Western European and Others**	**Western European and Others**	**Western European and Others**
2000–2003	7	129	206	252	526
2004–2007	5	29	82	99	156

(continued)

Table 2 (continued)

Period	*N*	Minimum	Median	Mean	Maximum
2008–2011	5	89	196	307	615
2012–2015	4	88	131	144	226
2016–2019	5	39	57	78	128
		All	**All**	**All**	**All**
2000–2003	16	129	445	601	1580
2004–2007	13	29	324	539	2830
2008–2011	45	56	465	930	8490
2012–2015	17	64	297	1825	23,500
2016–2019	43	17	125	380	7100
2000–2019	134	17	255	778	23,500

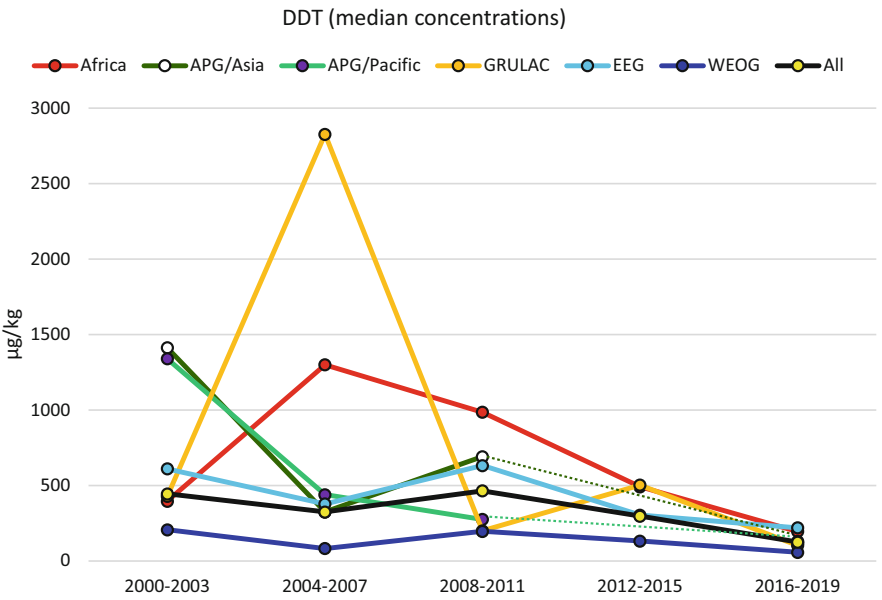

Fig. 1 Time trends for median concentrations of DDT in human milk (expressed as µg DDT complex/kg lipid) in the five UN regions (with split of the Asia-Pacific Group [APG] into the subgroups Asia and Pacific Islands; GRULAC, Group of Latin American and Caribbean Countries; EEG, Eastern European Group; WEOG, Western European and Others Group) in five periods between 2000 and 2019

reason: The number of countries participating from a certain UN region in a certain period varied considerably. As example, from 26 countries participating in the 2000–2003 period, 21 were from the Eastern European Group and the Western European and Others Group, whereas only five countries from the other groups (African; Asia-Pacific that is split into two subgroups Asia and Pacific Islands; Latin American and Caribbean) had participated. The proportion of countries from the UN

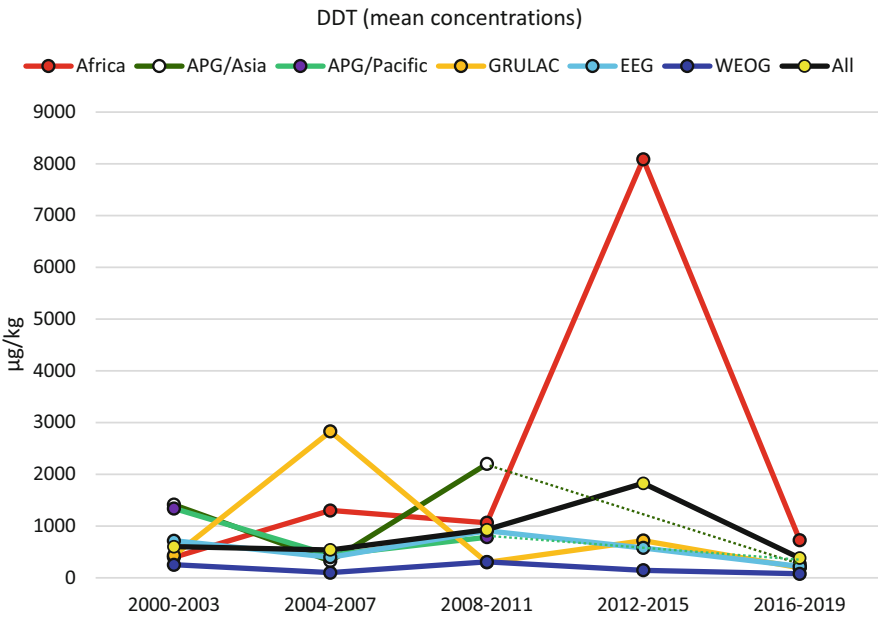

Fig. 2 Time trends for mean concentrations of DDT in human milk (expressed as µg DDT complex/kg lipid) in the five UN regions (with split of the Asia-Pacific Group [APG] into the subgroups Asia and Pacific Islands; GRULAC, Group of Latin American and Caribbean Countries; EEG, Eastern European Group; WEOG, Western European and Others Group) in five periods between 2000 and 2019

regions changed in the 2008–2011 period, when 37 of the 45 participating countries were from Africa, Asia, Latin America and the Caribbean, and similarly in 2016–2018, when 36 of the 43 countries were from these other groups (Malisch et al. 2023a). It is observed that it is difficult to draw general conclusions on time trends, especially when three or less countries in a region have participated in a certain period, and in particular if a country with a very high concentration participated in single period: This could have a considerable effect on median or mean concentrations for this group during the period.

The assessment of time trends based only on country-specific results of countries with repeated participation allows more certainty in drawing of conclusions, and therefore is optimal for the evaluation of the effectiveness for the purpose of Article 16. This evaluation (comprising also DDT) is published separately in Part IV (Malisch et al. 2023b).

3.1.2 African Group

Of all UN regions, Africa had the widest variation in contamination of human milk with DDT complex. Figure 3 illustrates these results for country results (with the five 4-year periods between 2000 and 2019 shown in different colors; for "country results" see Sect. 2.2). The lowest concentration (17 µg DDT complex/kg lipid)

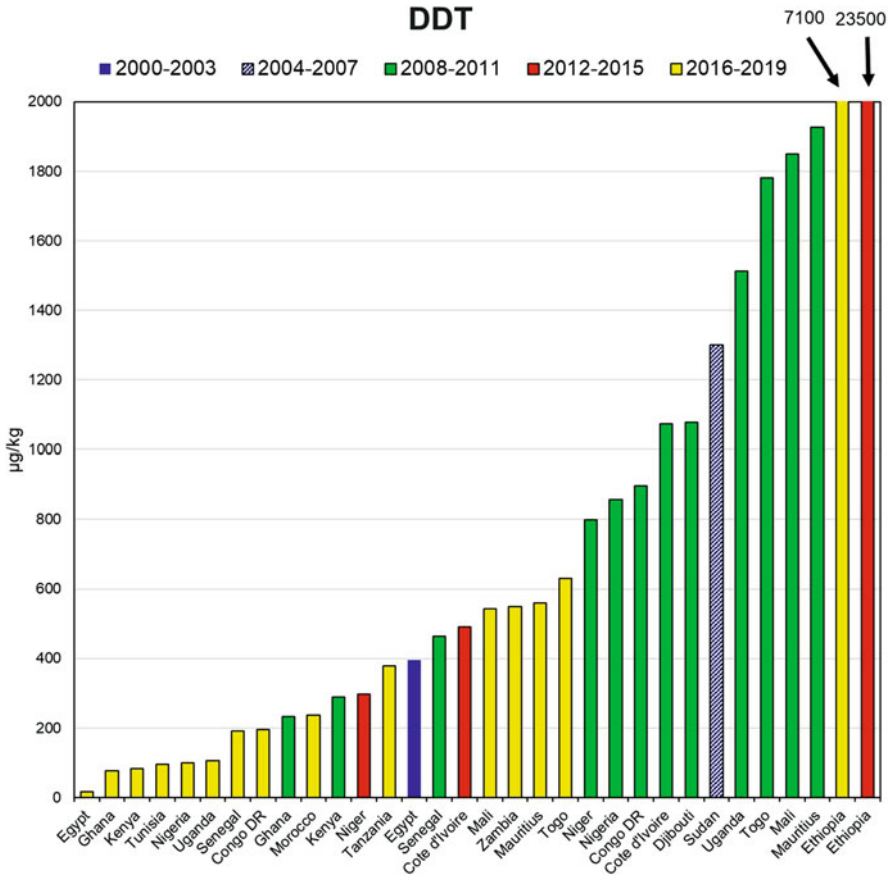

Fig. 3 Concentrations of DDT in human milk from countries in the African Group for the five periods from 2000–2019 (expressed as µg ∑ DDT complex/kg lipid)

was found in Egypt in 2019; the highest concentration of 23,500 µg DDT complex/kg lipid (or 23.5 mg DDT complex/kg lipid) found in Ethiopia in 2012. In this sample, about 50% of the DDT complex came from *p,p′*-DDE, 46% from *p,p′*-DDT and 4% from *o,p′*-DDT. This is indicative of more recent use and contamination probably due to public health use of DDT to combat mosquitos for malaria control, which is permitted under the Stockholm Convention with some constraints. These conclusions are in line with findings of high mean levels of DDT complex of 12,680 µg/kg lipid (calculated without correction factors) in human milk from Ethiopia collected in 2010 in three cities in areas where malaria is prevalent, and where annual spraying for malaria control was common. Between 55 and 71% of DDT complex was attributed to *p,p′*-DDT which was indicative of the continued use of DDT at that time. A number of measures were recommended to reduce the levels of DDT exposure (Gebremichael et al. 2013). As a result of the implementation of

these measures, the 2019 sample from Ethiopia showed a considerable downward trend with 7100 μg DDT complex/kg lipid. Importantly, in the 2019 sample *p, p′*-DDE contributed nearly 80% and *p,p′*-DDT 20% to DDT complex.

3.1.3 Asia-Pacific Group

Asia Subgroup

Figure 4 illustrates the DDT results with the period of participation between 2000 and 2019 indicated for the Asian countries of the Asia-Pacific Group. A wide range of DDT concentrations was found, with the lowest concentration in Mongolia in 2018 (45 μg DDT complex/kg lipid) and the highest concentration in Tajikistan in

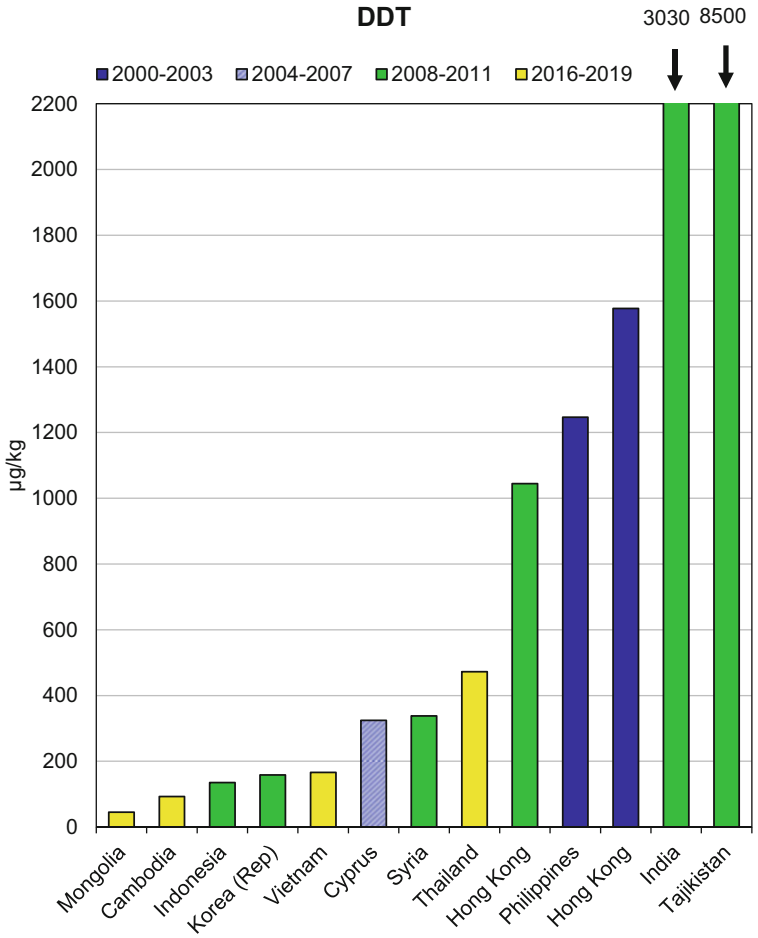

Fig. 4 Concentrations of DDT in human milk from countries in the Asia-Pacific Group/Asia subgroup for the five periods from 2000 to 2019 (expressed as μg ∑DDT complex/kg lipid)

2009 (8490 μg DDT complex/kg lipid). In this sample with (in other units) about 8.5 mg DDT complex/kg lipid, nearly 98% of the contribution to the sum parameter "DDT complex" came from *p,p′*-DDE, which suggests legacy contamination through past exposure.

Samples from earlier rounds (UNEP 2013) had higher levels than countries participating in the 2016–2019 round. Hong Kong SAR of China participated twice and showed a decrease to the 2009 level of 1040 μg DDT complex/kg lipid (median of four samples from different subgroups) from the 2002 level of 1580 μg DDT complex/kg lipid (median of ten samples from different population subgroups [Hui et al. 2008]).

Pacific Islands Subgroup

Figure 5 illustrates the DDT results with the period of participation between 2000 and 2019 indicated for the Pacific Islands countries of the Asia-Pacific Group. A

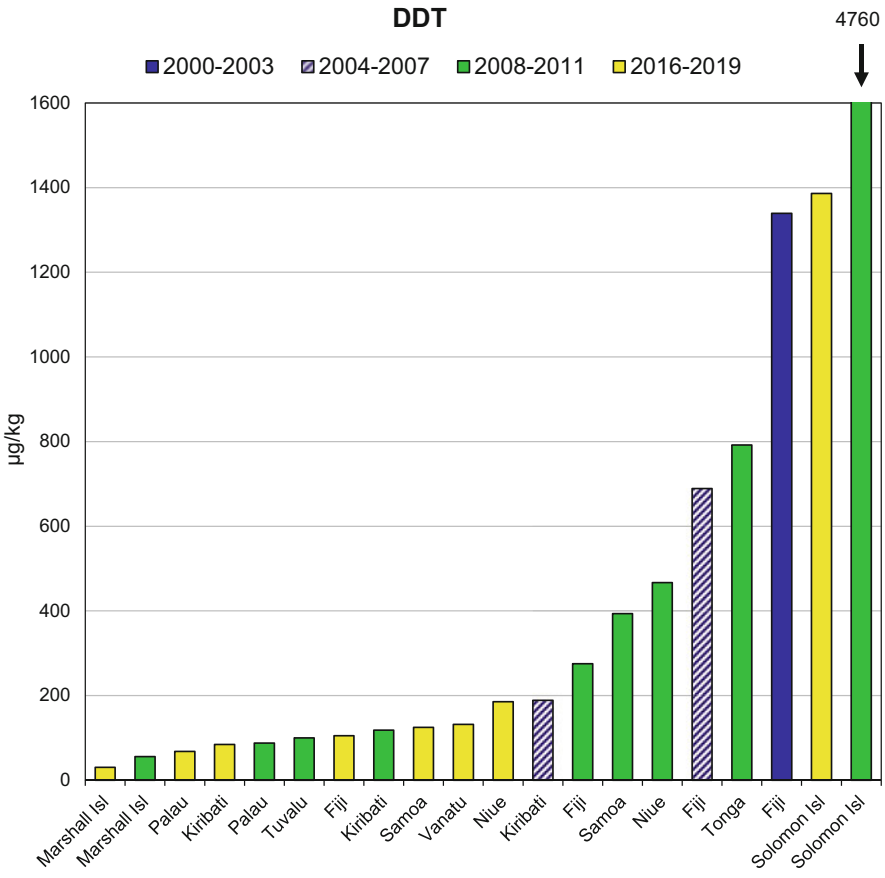

Fig. 5 Concentrations of DDT in human milk from countries in the Asia-Pacific Group/Pacific Islands subgroup for the five periods from 2000 to 2019 (expressed as μg DDT complex/kg lipid)

wide range of DDT concentrations was found, with the lowest concentration in the Marshall Islands in 2019 (31 μg DDT complex/kg lipid) and the highest concentration in the Solomon Islands in 2011 (4760 μg DDT complex/kg lipid). In this sample with (in other units) 4.8 mg DDT complex/kg lipid, 91% of the contribution to the sum parameter "DDT complex" came from *p,p′*-DDE. With 1390 μg DDT complex/kg lipid, the sample of 2019 from the Solomon Islands had considerably lower DDT levels, with a contribution of 95% from *p,p′*-DDE.

Results of four participations of Fiji (in 2001, 2006, 2011, and 2019) show a considerable downwards trend from initially 1340 μg DDT complex/kg lipid to 105 μg/kg lipid, with a contribution of *p,p′*-DDE to DDT complex increasing gradually from 82% in 2001 to 96% in 2019 as indication of an old contamination (with decreasing absolute *p,p′*-DDE levels from 990 to 91 μg/kg lipid).

3.1.4 Group of Latin American and Caribbean Countries (GRULAC)

Figure 6 illustrates the DDT results with the period of participation between 2000 and 2019 indicated for Latin American and Caribbean countries. A wide range of DDT concentrations was found, with the lowest concentration in Uruguay in 2019 (46 μg DDT complex/kg lipid). The highest concentration was observed in Haiti in 2005 (2830 μg DDT complex/kg lipid), but more recent samples showed a considerable downward trend to 574 μg DDT complex/kg lipid in 2011 and 263 μg DDT complex/kg in 2015.

Due to its large size and population of over 200 million, Brazil submitted 10 pooled samples from various regions in 2001 and 2002 and three pooled national samples in 2012. Brazil is an example of the need for flexible criteria in collecting a representative sample for a country. According to the protocol, one pooled sample for countries with populations of fewer than 50 million is requested. Countries with populations well over 50 million (or with sufficient resources) were encouraged to prepare a second pooled sample (or more) if feasible.

3.1.5 Eastern European Group

Figure 7 illustrates the DDT results for the period of 2000–2019 for countries from the Eastern European Group. The DDT concentrations ranged from 64 μg DDT complex/kg lipid (Croatia, 2014) to 1820 μg DDT complex/kg lipid (Moldova, 2009), which showed a decrease to 1180 μg DDT complex/kg lipid in 2015. Results for the Czech Republic in 2001, 2006, 2014, and 2019 show a downward trend from initially 461 μg DDT complex/kg lipid to 192 μg DDT complex/kg lipid.

3.1.6 Western European and Others Group (WEOG)

On average, countries of the Western European and Others Group (WEOG), which includes Australia, Israel, New Zealand, and the USA (being informally a member) as "Others," had the lowest DDT concentrations, with a range between 29 μg DDT complex/kg lipid (Finland, 2007) and 615 μg DDT complex/kg (Australia, 2010) and a downward trend in Australia to 227 μg DDT complex/kg lipid in 2013 (Fig. 8). The comparably low concentrations in WEOG countries are likely due to early bans on the use of DDT in agriculture implemented in most of these countries.

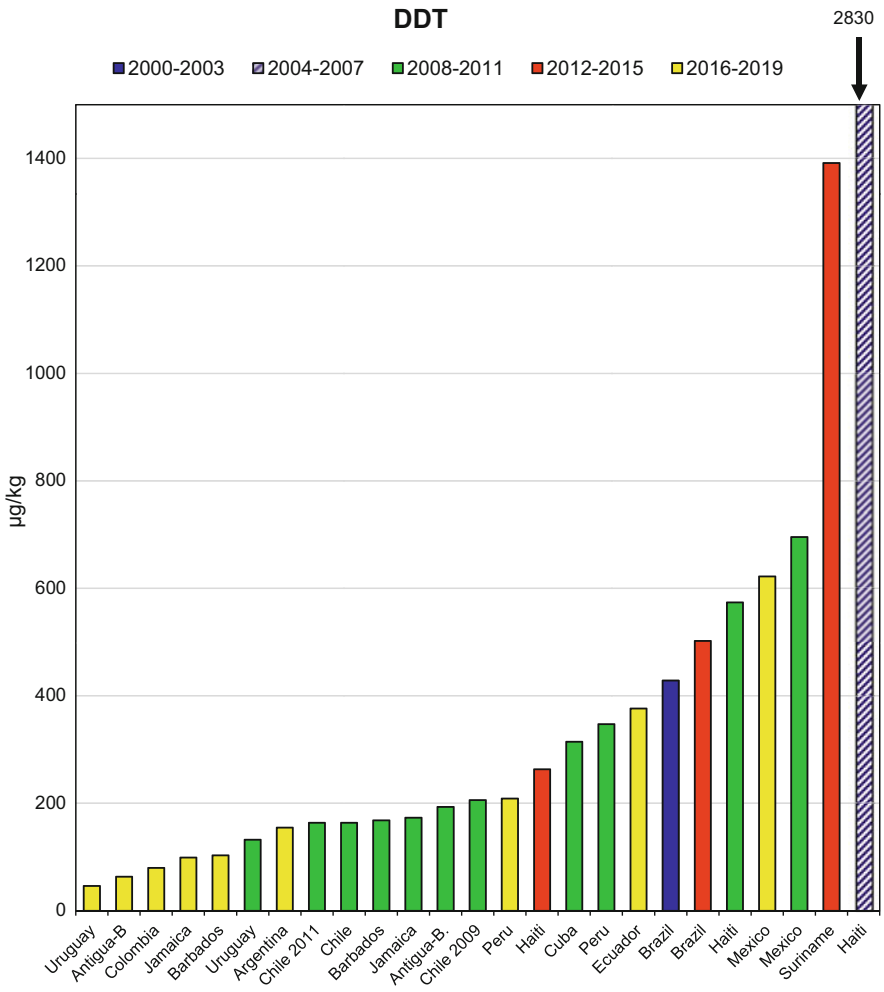

Fig. 6 Concentrations of DDT in human milk from Latin American and Caribbean countries for the five periods from 2000 to 2019 (expressed as µg DDT complex/kg lipid)

The gradual downward trend of the background contamination over the whole-time span between 2000 and 2019 can be illustrated by Germany. After the ban of DDT in the Federal Republic of Germany in 1972 and in the German Democratic Republic at the end of the 1980s (Umweltbundesamt 2021), background levels were at 161 µg DDT complex/kg in 2002 and went further down to 57 µg DDT complex/kg lipid in 2019.

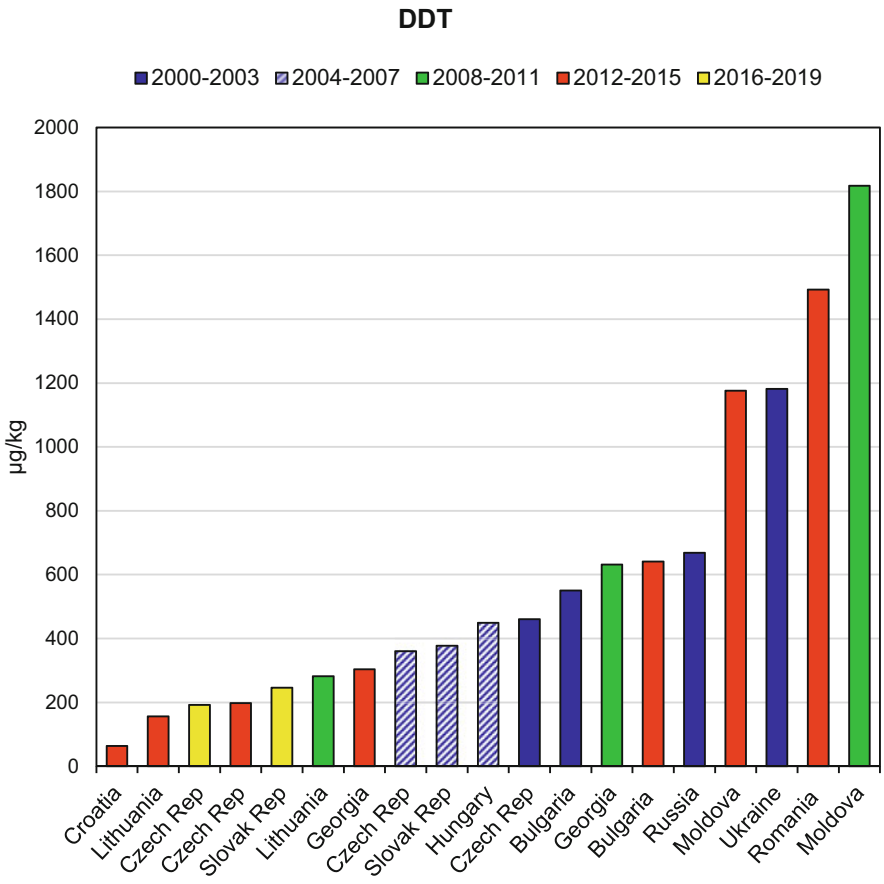

Fig. 7 Concentrations of DDT in human milk from countries in the Eastern European Group for the five periods from 2000 to 2019 (expressed as µg DDT complex/kg lipid)

4 Hexachlorocyclohexanes (Alpha-HCH, Beta-HCH, and Gamma-HCH)

Technical-grade hexachlorocyclohexane (HCH) is a mixture mainly of three isomers comprising about 65–70% alpha-HCH, 7–20% beta-HCH, and 14–15% gamma-HCH. Note that only gamma-HCH (lindane) has insecticidal properties. Due to metabolization, mainly beta-HCH accumulates in humans (Fürst 2023; Fång et al. 2015). Alpha-HCH, beta-HCH, and gamma-HCH are the most important isomers and were listed in 2009 in Annex A (for elimination) by the Stockholm Convention (UNEP 2009). In contrast, the minor components delta-HCH (6–10%) and epsilon-HCH (1–2%) were not listed. Therefore, in this article, HCH complex is defined as the sum of alpha-HCH, beta-HCH, and gamma-HCH.

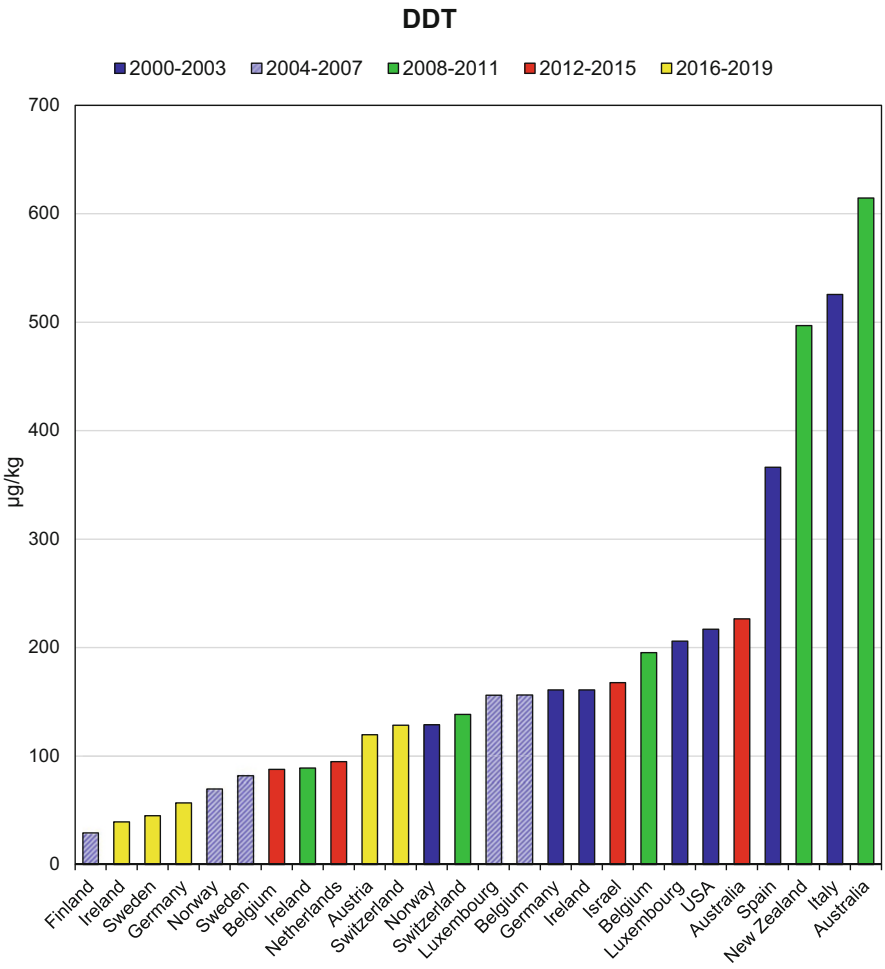

Fig. 8 Concentrations of DDT in human milk from countries in the Western European and Others Group for the five periods from 2000 to 2019 (expressed as µg DDT complex/kg lipid)

As a result of the metabolization of hexachlorocyclohexanes in humans, the concentrations of alpha-HCH and gamma-HCH were below the limit of quantification in most human milk samples (<0.5 µg/kg lipid) with a median of quantifiable residues of about 1 µg/kg lipid and maxima of 10.5 µg/kg for alpha-HCH and 16 µg/kg for gamma-HCH. In most cases with HCH complex concentrations above 10 µg/kg lipid, about 95–100% of this sum parameter are attributed to beta-HCH. Therefore, only the ranges found for beta-HCH are discussed in more detail in the following subsection. The detailed results for alpha-HCH, beta-HCH, and gamma-HCH are available at the POPs Global Monitoring Plan Data Warehouse (Global Monitoring Plan Data Warehouse 2020).

4.1 General Comparison of Beta-HCH Ranges

The comparison of the ranges of beta-HCH among UN regions found in the five periods over 20 years is compiled in Table 3. Great differences were found, with a minimum of <0.5 μg beta-HCH /kg lipid found in few countries and a maximum of 1020 μg beta-HCH /kg lipid found in 2002 in the Asia subregion of the Asia-Pacific Group. The median of 134 country results (see Sect. 2.2) was 5.9 μg beta-HCH /kg lipid.

The wide range of concentrations raised the question whether this reflects different patterns of usage or production. Historical production of technical HCH and lindane occurred in many European countries, including the Czech Republic, Spain, France, Germany, United Kingdom, Italy, Romania, Bulgaria, Poland, and Turkey, and took place mainly from 1950 or earlier and stopped in 1970 to the 1990s. According to a research by IHPA, technical HCH and lindane have also been produced in other countries including Albania, Argentina, Austria, Azerbaijan, Brazil, China, Ghana, Hungary, India, Japan, Russia, Slovakia and the United States. Exact information is difficult to obtain, as many countries do not keep records of historical pesticides production, sales and usage or the industry considers this to be proprietary information (UNEP 2006a). In its risk profile on beta-HCH, POPRC reviewed concentrations in breast milk and found reports of very high concentrations (up to 800 ng/g) in Russia, Ukraine, and Romania (UNEP 2006b). In 2009, alpha-HCH, beta-HCH, and gamma-HCH were listed to the Stockholm Convention (UNEP 2009). The global overview of POPs data in human milk compiled in a review of scientific publications between 1995 and 2011 shows a wide range of beta-HCH concentrations, with ∑HCH concentrations up to 22,000 ng/g fat (Fång et al. 2015).

As for DDT, conclusions on **time trends** for beta-HCH in the different regions cannot easily be drawn. As illustrated by Fig. 9, the time trends of the *median* concentrations in the UN regions are not consistent. For the median of the samples in all UN regions, a decrease of 91% was found between the 2000–2003 period and the 2016–2019 period. A considerable decrease is also seen in the individual UN regions if the median of the beta-HCH concentrations of the 2000–2003 period is compared to the 2016–2019 period. However, in the three rounds in-between, considerable variations were observed, notably in Asian and in the Eastern European countries starting with an initial downward trend from 2000–2003 to 2004–2007. This, however, is followed by an increase in the period 2008–2011 before the concentrations decrease again to 2016–2019.

The picture using the *mean* concentrations instead of median looks quite comparable with no significant maxima in other periods (Fig. 10). As explained for DDT, a more precise method to derive time trends is the evaluation only of country-specific results of countries with repeated participation. This allows more certainty in drawing of conclusions on temporal trends. Therefore, this assessment of these more precise time trends for beta-HCH is part of a special chapter in Part IV (Malisch et al. 2023b).

Table 3 Minimum, median, mean, and maximum concentrations of beta-HCH in human milk (µg beta-HCH /kg lipid) in the five UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands) for the five periods between 2000 and 2019 based on 134 country results (see Sect. 2.2) with the number of country results [*N*] in the respective period

Period	*N*	Minimum	Median	Mean	Maximum
		African	**African**	**African**	**African**
2000–2003	1	51.3	51.3	51.3	51.3
2004–2007	1	36.5	36.5	36.5	36.5
2008–2011	12	1.8	7.7	13.7	48.3
2012–2015	3	<0.5	1.0	6.6	18.2
2016–2019	15	<0.5	2.2	4.3	19.7
		Asia-Pacific/ Asia	**Asia-Pacific/ Asia**	**Asia-Pacific/ Asia**	**Asia-Pacific/ Asia**
2000–2003	2	8.4	516	516	1020
2004–2007	1	21.0	21.0	21.0	21.0
2008–2011	6	3.7	128	236	845
2012–2015	0				
2016–2019	4	0.6	2.0	11.6	41.6
		Asia-Pacific/ Pacific Islands	**Asia-Pacific/ Pacific Islands**	**Asia-Pacific/ Pacific Islands**	**Asia-Pacific/ Pacific Islands**
2000–2003	1	5.8	5.8	5.8	5.8
2004–2007	2	2.7	4.4	4.4	6.1
2008–2011	9	1.3	2.6	2.7	4.9
2012–2015	0				
2016–2019	8	< 0.5	1.3	1.8	6.5
		Latin American and Caribbean	**Latin American and Caribbean**	**Latin American and Caribbean**	**Latin American and Caribbean**
2000–2003	1	24.6	24.6	24.6	24.6
2004–2007	1	<0.5	<0.5	<0.5	<0.5
2008–2011	10	0.7	4.8	7.9	29.7
2012–2015	3	<0.5	2.9	7.1	17.9
2016–2019	9	0.7	2.9	4.8	15.9
		Eastern European	**Eastern European**	**Eastern European**	**Eastern European**
2000–2003	4	26.0	104	129	279
2004–2007	3	8.5	18.3	15.6	20.1
2008–2011	3	16.6	92.6	195	476
2012–2015	7	3.8	25.1	94.0	375
2016–2019	2	2.3	2.4	2.4	2.5
		Western European and Others	**Western European and Others**	**Western European and Others**	**Western European and Others**
2000–2003	7	10.8	22.9	29.9	58.8
2004–2007	5	3.9	6.6	9.2	17.7
2008–2011	5	4.0	8.9	9.4	17.2

(continued)

Table 3 (continued)

Period	*N*	Minimum	Median	Mean	Maximum
2012–2015	4	4.3	8.9	9.2	14.7
2016–2019	5	1.9	3.6	9.8	23.4
		All	**All**	**All**	**All**
2000–2003	16	5.8	25.3	115	1020
2004–2007	13	<0.5	10.2	13.3	36.5
2008–2011	45	0.7	5.7	49.4	845
2012–2015	17	<0.5	14.7	49.0	375
2016–2019	43	<0.5	2.4	5.5	41.6
2000–2019	134	<0.5	5.9	39.3	1020

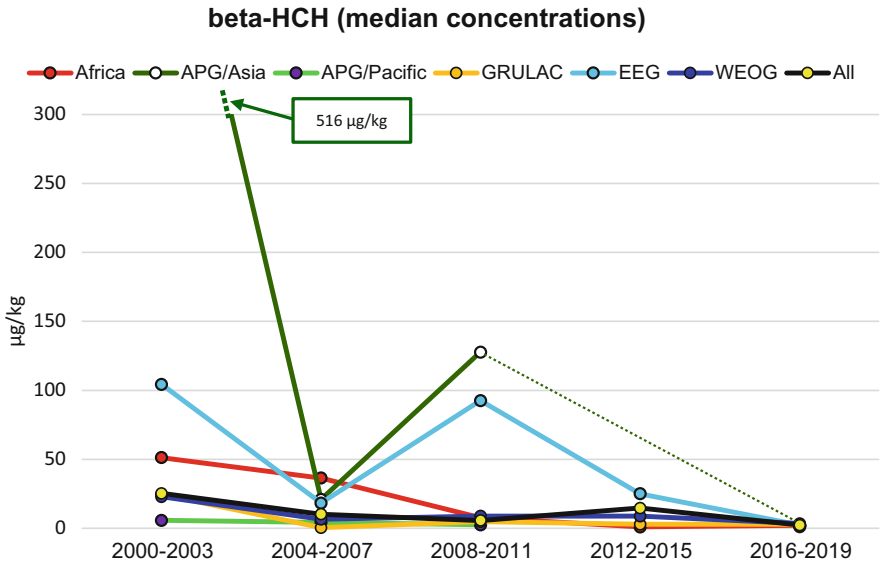

Fig. 9 Time trends for median concentrations of beta-HCH in human milk (expressed as µg beta-HCH /kg lipid) in the five UN regions (with split of the Asia-Pacific Group [APG] into the subgroups Asia and Pacific Islands; GRULAC, Group of Latin American and Caribbean Countries; EEG, Eastern European Group; WEOG, Western European and Others Group) in five periods between 2000 and 2019

4.2 African Group

The range of beta-HCH in samples from African countries varied between <0.5 µg/kg lipid in three countries and 51.3 µg/kg lipid found in Egypt in 2002 (Fig. 11). In Egypt, a considerable downward trend was observed when 19.7 µg/kg lipid was found in 2019. In both samples from this country, 94% of the HCH complex was from beta-HCH.

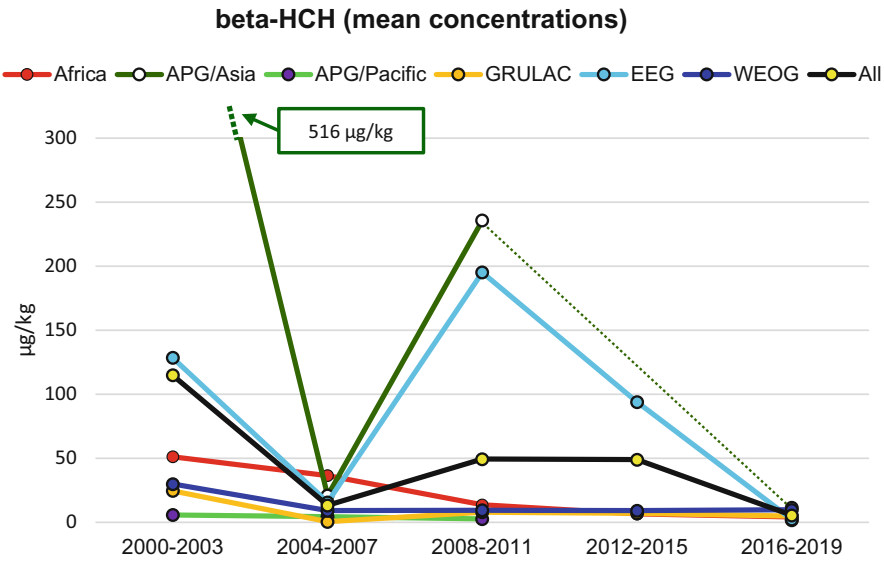

Fig. 10 Time trends for mean concentrations of beta-HCH in human milk (expressed as µg beta-HCH /kg lipid) in the five UN regions (with split of the Asia-Pacific Group [APG] into the subgroups Asia and Pacific Islands; GRULAC, Group of Latin American and Caribbean Countries; EEG, Eastern European Group; WEOG, Western European and Others Group) in five periods between 2000 and 2019

In contrast, 16 µg gamma-HCH /kg lipid was found in the sample from Senegal of 2009, corresponding to 25% of HCH complex, with 48.3 µg beta-HCH /kg lipid. Here, the contribution of gamma-HCH to HCH complex fell to 13% until 2018, and the beta-HCH concentration fell to 11.7 µg/kg.

4.3 Asia-Pacific Group

4.3.1 Asia Subgroup

Figure 12 illustrates the beta-HCH results for the period 2000–2019 for the Asian countries of the Asia-Pacific Group. A wide range of concentrations was found. Samples from previous rounds (UNEP 2013) had higher levels than samples from countries participating in the 2016–2019 round. The lowest concentration was found in the sample from Cambodia collected in 2019 (0.6 µg beta-HCH /kg lipid) and the highest concentration was in the sample from Hong Kong SAR of China in 2002 (1020 µg beta-HCH /kg lipid as the median of ten pooled samples [Hedley et al. 2010]). Hong Kong participated twice, with a considerable decrease found in the 2009 sample, where the concentration was 290 µg beta-HCH/kg lipid (median of four samples from different population groups).

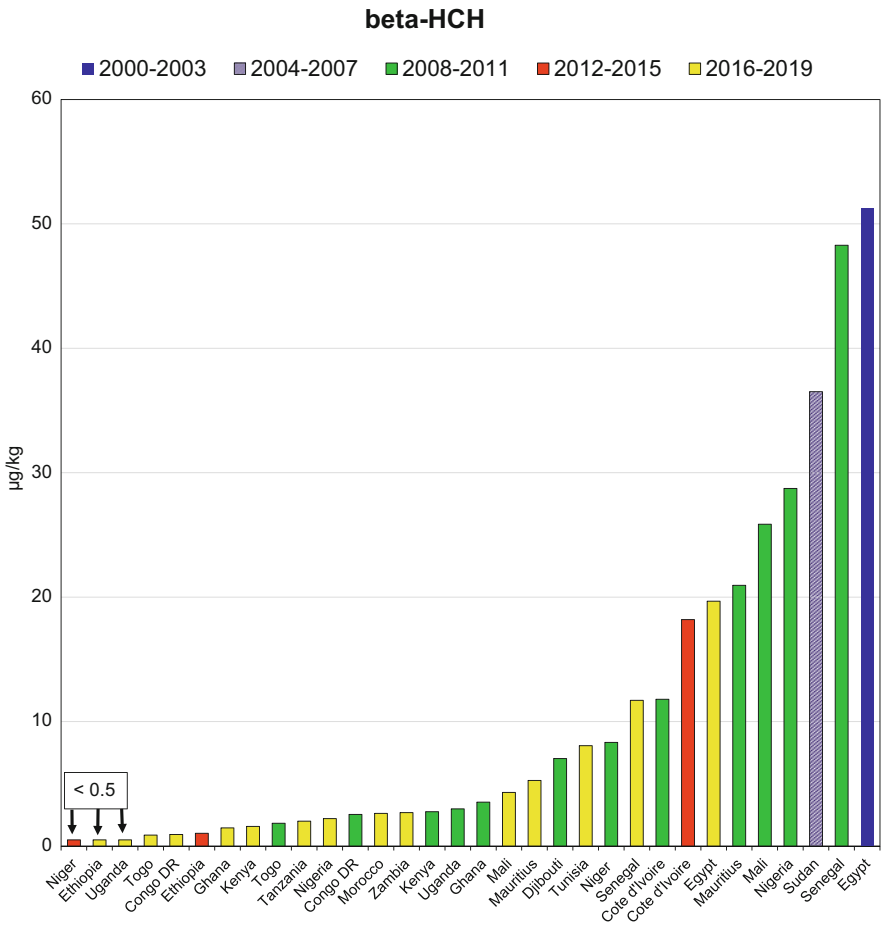

Fig. 11 Concentrations of beta-HCH in human milk (µg beta-HCH /kg lipid) from countries in the African Group for the five periods from 2000 to 2019

4.3.2 Pacific Islands Subgroup

Figure 13 illustrates the beta-HCH results for the Pacific Islands countries of the Asia-Pacific Group. All countries in all periods had beta-HCH concentrations in the range of low background contamination between <0.5 µg/kg lipid and 6.5 µg/kg lipid. Overall, countries of this region had the lowest beta-HCH concentrations in human milk.

4.4 Group of Latin American and Caribbean Countries (GRULAC)

Figure 14 illustrates the beta-HCH results for Latin American and Caribbean countries. The results ranged between <0.5 µg beta-HCH /kg lipid (Haiti, 2004

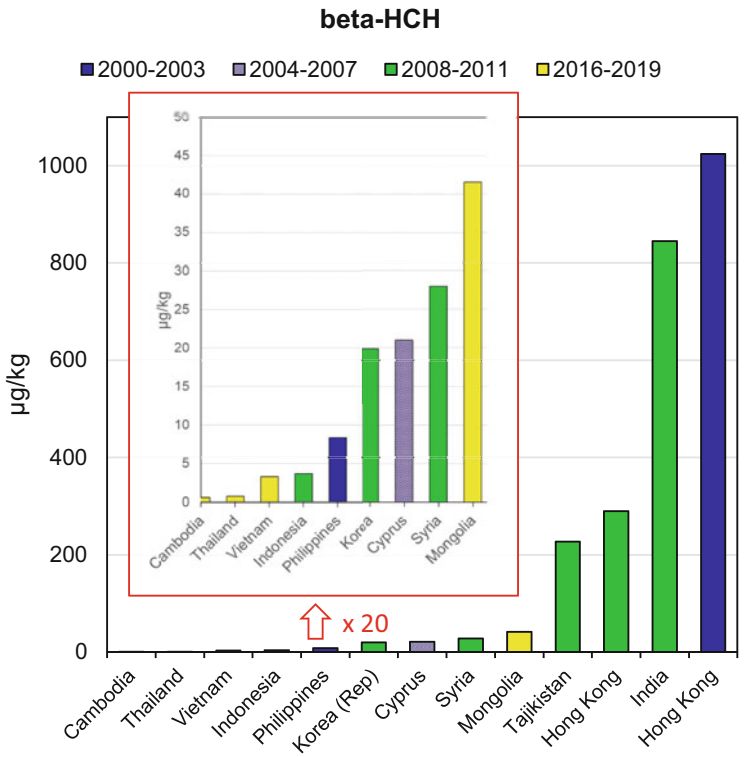

Fig. 12 Concentrations of beta-HCH in human milk (µg beta-HCH /kg lipid) from countries in the Asia-Pacific Group/Asia subgroup for the five periods from 2000 to 2019

and 2015) and 29.7 µg beta-HCH /kg lipid (Uruguay, 2009). In countries with beta-HCH concentrations above 5 µg/kg as found in samples submitted before 2011 (Uruguay, 2009; Brazil, 2001 and 2002; Peru, 2011; Chile, 2008; Barbados, 2010), a considerable downward trend was observed until 2019.

4.5 Eastern European Group

Figure 15 illustrates the beta-HCH results for countries of the Eastern European Group. A wide range of concentrations was observed between the low background contamination as found in 2019 in the Slovak Republic (2.3 µg beta-HCH /kg lipid) and the Czech Republic (2.5 µg beta-HCH /kg lipid), and the maximum of 476 µg beta-HCH /kg lipid found in 2009 in Moldova. In Moldova, these concentrations decreased considerably to 150 µg beta-HCH/kg lipid in 2015. The Czech Republic participated four times between 2000 and 2019; here, a continuous downward trend was observed from 26 µg beta-HCH /kg lipid in 2001 to 2.5 µg beta-HCH /kg lipid in 2019.

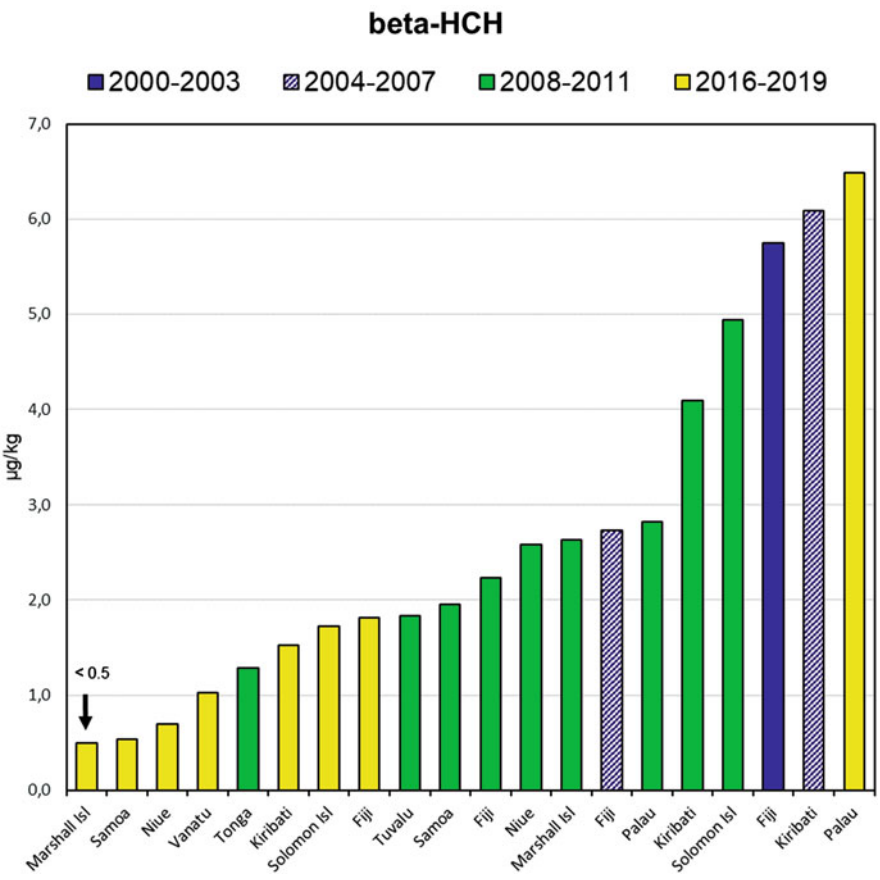

Fig. 13 Concentrations of beta-HCH in human milk (µg beta-HCH/kg lipid) from countries in the Asia-Pacific Group/Pacific Islands subgroup for the five periods from 2000 to 2019

4.6 Western European and Others Group (WEOG)

Figure 16 illustrates the beta-HCH results for countries of the Western European and Others Group. The concentrations ranged from 1.9 µg beta-HCH /kg lipid (Sweden, 2019) to 58.8 µg beta-HCH /kg lipid (Spain, 2002, median of three pooled samples). The three highest concentrations were found in countries participating in the 2000–2003 period and the three lowest concentrations in the 2016–2019 period.

The gradual downward trend of the background contamination over the whole span of time between 2000 and 2019 is illustrated by Ireland: Background levels of beta-HCH were at 24 µg/kg in 2002 and went down 17.2 µg/kg lipid in 2010 and finally to 3.6 µg/kg lipid in 2019.

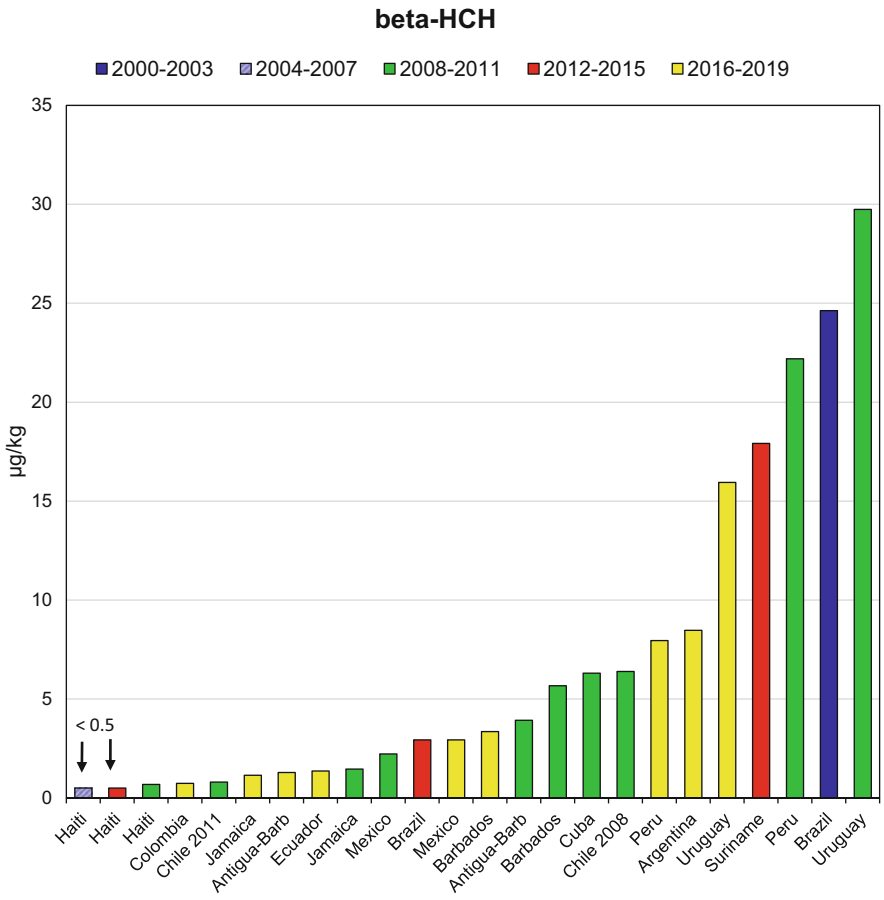

Fig. 14 Concentrations of beta-HCH (µg beta-HCH /kg lipid) in human milk from Latin American and Caribbean countries for the five periods from 2000 to 2019

5 Hexachlorobenzene (HCB)

Hexachlorobenzene (HCB) belongs to the group of the "12 Initial POPs" under the Stockholm Convention. Production and use of HCB, e.g., formerly used as a fungicide for seed treatment or for various technical purposes, is prohibited by listing HCB in Annex A (Elimination) of the convention. Furthermore, HCB can be formed as an unintentional by-product, e.g., in certain thermal processes. Therefore, HCB is also listed in Annex C (Unintentional production) for minimization and, where feasible, ultimate elimination of unintentional releases (UNEP 2001). HCB is chemically and physiologically stable, and thus persists in the environment and in biological systems. It is also highly lipophilic, which results in its biomagnification in the food chain and bioaccumulation in fatty tissues of animals

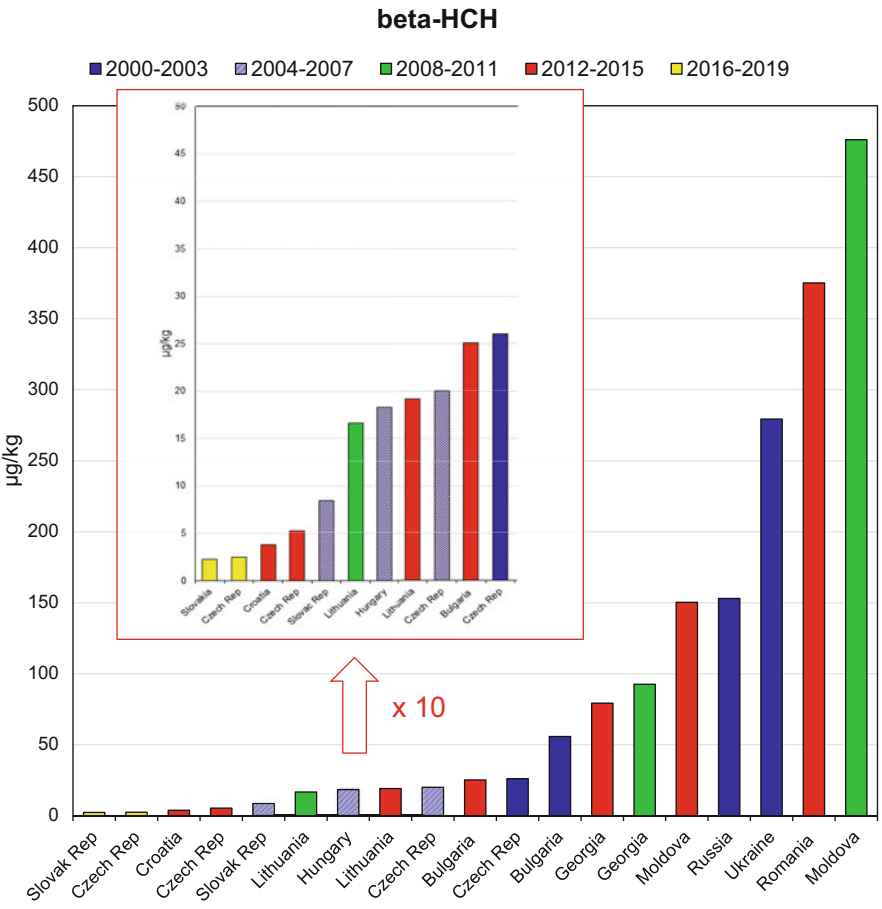

Fig. 15 Concentrations of beta-HCH in human milk (µg beta-HCH /kg lipid) from countries in the Eastern European Group for the five periods from 2000 to 2019

and humans (Fürst 2023). The global overview of POPs data in human milk compiled in a review of scientific publications between 1995 and 2011 shows a wide range of HCB concentrations up to the range of about 1000 ng/g fat (Fång et al. 2015).

5.1 General Comparison of HCB Ranges

The comparison of the ranges of HCB among UN regions found in the five periods over 20 years is compiled in Table 4. The maximum levels and therefore the ranges were much lower than found for DDT and beta-HCH, with a minimum of about 1–2 µg/kg lipid found in some countries and a maximum of 154 µg/kg lipid found in 2009 in an Eastern European country. The median of 134 country results (see Sect.

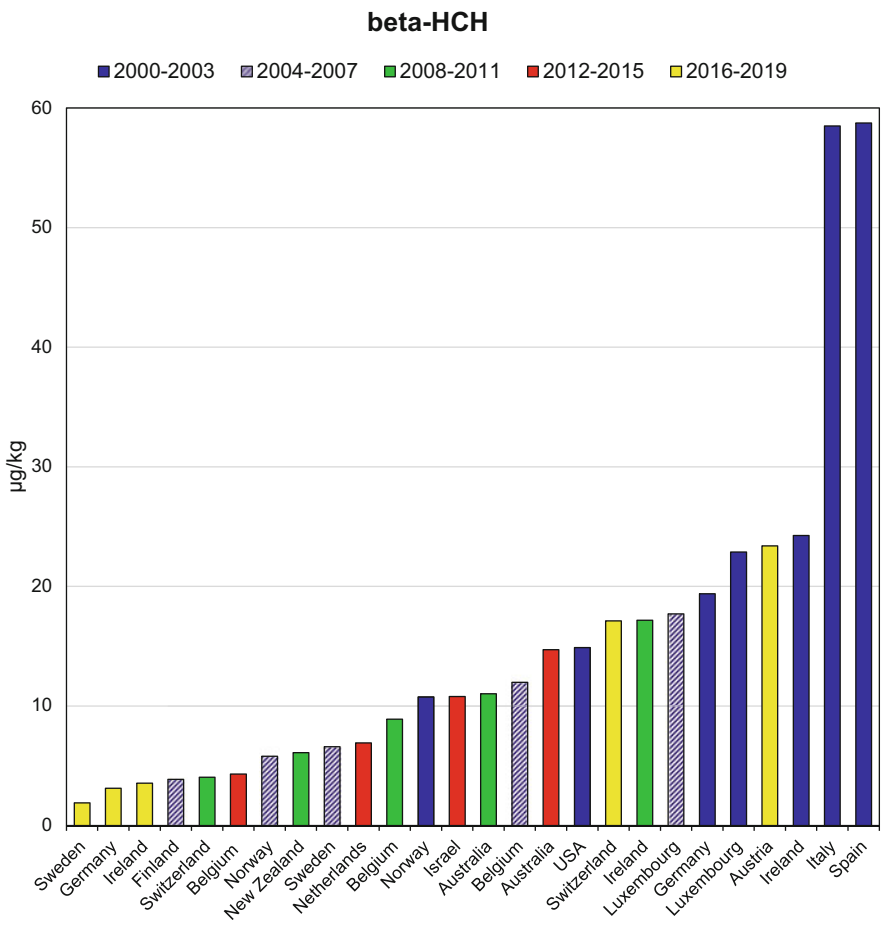

Fig. 16 Concentrations of beta-HCH in human milk (µg beta-HCH /kg lipid) from countries in the Western European and Others Group for the five periods from 2000 to 2019

2.2) was 5.1 µg/kg lipid. Samples from all countries from Africa were at all times in this low background range below 5 µg/kg lipid, and samples from many countries from the Pacific Islands and Latin America and the Caribbean were in the same low range. The highest HCB concentrations were found in the Eastern European Group.

In general, concentrations of HCB show a downward trend globally: The median of all samples decreases from the 2000–2003 period (16 countries, 16.4 µg/kg lipid) to the 2016–2019 period (43 countries, 3.3 µg/kg lipid) by about 80%. Of particular interest are time trends in regions with higher initial concentrations. Figure 17 illustrates the decline of the *median* HCB concentrations in various groups. In the Eastern European Group, the median HCB concentration falls from the 2000–2003 period to the 2012–2015 period and then levels out around 13 µg/kg lipid. In countries of the Western European and Others Group, a downward trend is also

Table 4 Minimum, median, mean, and maximum concentrations of HCB in human milk (expressed as μg/kg lipid) in the five UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands) for the five periods between 2000 and 2019 based on 134 country results (see Sect. 2.2) with the number of country results [*N*] in the respective period

Period	*N*	Min	Median	Mean	Max
		African	**African**	**African**	**African**
2000–2003	1	3.8	3.8	3.8	3.8
2004–2007	1	3.2	3.2	3.2	3.2
2008–2011	12	1.6	2.5	2.8	5.0
2012–2015	3	1.8	1.8	2.3	3.4
2016–2019	15	1.3	2.3	2.4	3.7
		Asia-Pacific/ Asia	**Asia-Pacific/ Asia**	**Asia-Pacific/ Asia**	**Asia-Pacific/ Asia**
2000–2003	2	3.3	11.3	11.3	19.3
2004–2007	1	14.2	14.2	14.2	14.2
2008–2011	6	2.8	6.8	9.3	25.8
2012–2015	0				
2016–2019	4	2.5	3.8	11.0	34.0
		Asia-Pacific/ Pacific Islands	**Asia-Pacific/ Pacific Islands**	**Asia-Pacific/ Pacific Islands**	**Asia-Pacific/ Pacific Islands**
2000–2003	1	3.6	3.6	3.6	3.6
2004–2007	2	3.1	3.1	3.1	3.2
2008–2011	9	3.6	6.0	6.9	15.9
2012–2015	0				
2016–2019	8	2.1	2.7	3.0	5.0
		Latin American and Caribbean	**Latin American and Caribbean**	**Latin American and Caribbean**	**Latin American and Caribbean**
2000–2003	1	4.4	4.4	4.4	4.4
2004–2007	1	2.8	2.8	2.8	2.8
2008–2011	10	1.4	4.7	6.5	14.1
2012–2015	3	1.0	1.4	2.2	4.3
2016–2019	9	3.0	4.5	4.8	7.1
		Eastern European	**Eastern European**	**Eastern European**	**Eastern European**
2000–2003	4	12.0	58.3	51.1	76.0
2004–2007	3	12.0	46.6	35.3	47.4
2008–2011	3	15.1	31.1	66.6	154
2012–2015	7	7.6	13.3	21.7	46.7
2016–2019	2	12.5	12.8	12.8	13.0
		Western European and Others	**Western European and Others**	**Western European and Others**	**Western European and Others**
2000–2003	7	6.6	19.5	27.7	71.5
2004–2007	5	2.8	15.0	11.9	17.8
2008–2011	5	5.1	9.0	8.5	12.6

(continued)

Table 4 (continued)

Period	*N*	Min	Median	Mean	Max
2012–2015	4	5.6	7.9	8.1	10.8
2016–2019	5	6.5	10.4	10.3	14.9
		All	**All**	**All**	**All**
2000–2003	16	3.3	16.4	27.0	76.0
2004–2007	13	2.8	12.0	14.8	47.4
2008–2011	45	1.4	5.3	10.2	154
2012–2015	17	1.0	7.6	11.6	46.7
2016–2019	43	1.3	3.3	5.2	34.0
2000–2019	134	1.0	5.1	11.2	154

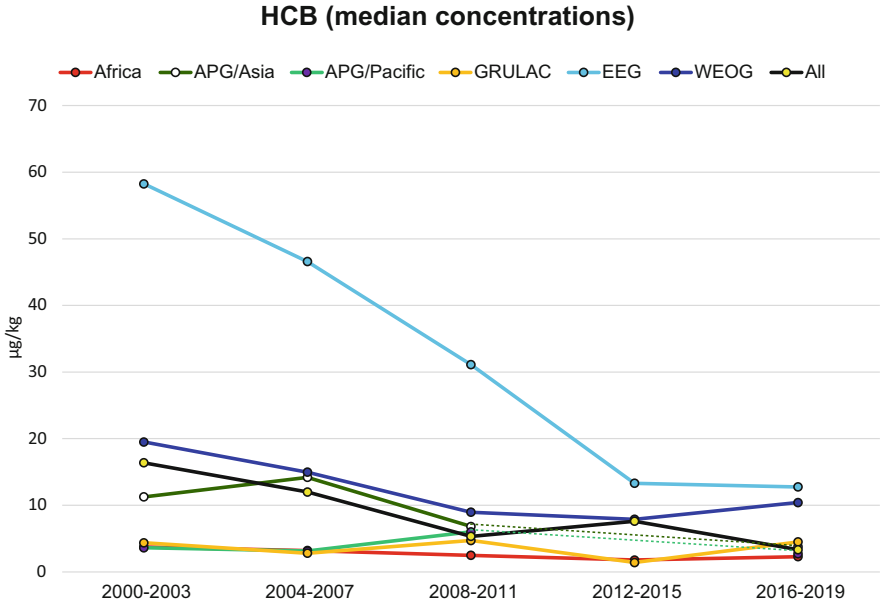

Fig. 17 Time trends for median concentrations of HCB in human milk (µg/kg lipid) in the five UN regions (with split of the Asia-Pacific Group [APG] into the subgroups Asia and Pacific Islands; GRULAC, Group of Latin American and Caribbean Countries; EEG, Eastern European Group; WEOG, Western European and Others Group) in five periods between 2000 and 2019

observed from the 2000–2003 period to the 2008–2011 period and then leveling out at about 10 µg/kg lipid. In other groups, the background contamination levels out at concentrations up to 5 µg HCB /kg lipid (limit of quantification: 0.5 µg/kg lipid).

The picture using the *mean* concentrations instead of median looks as if there was a maximum in the Eastern European Group in the 2008–2011 period (Fig. 18). However, this is caused by a high HCB concentration found in the pooled sample of one country submitted at that time. As explained before, a more precise method to derive time trends is the evaluation of results of countries only with repeated

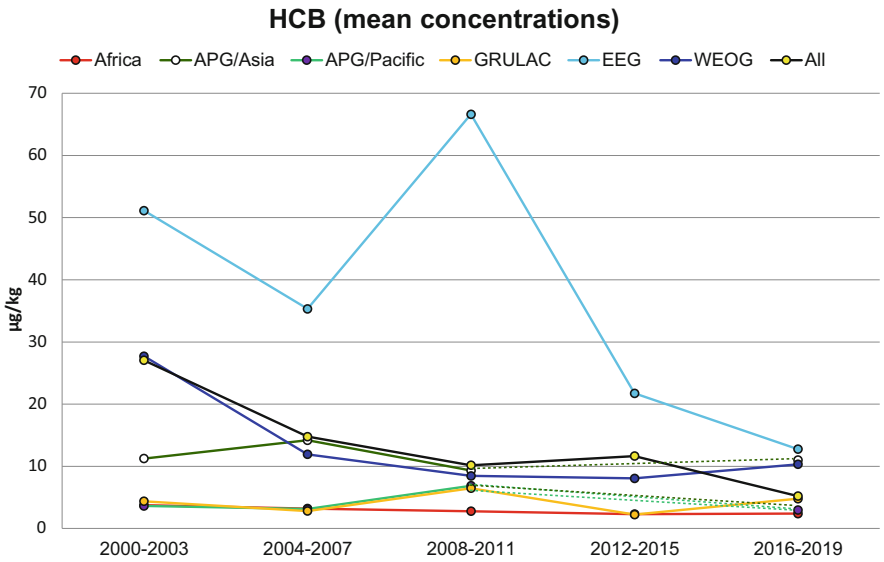

Fig. 18 Time trends for mean concentrations of HCB in human milk (µg/kg lipid) in the five UN regions (with split of the Asia-Pacific Group [APG] into the subgroups Asia and Pacific Islands; GRULAC, Group of Latin American and Caribbean Countries; EEG, Eastern European Group; WEOG, Western European and Others Group) in five periods between 2000 and 2019

participation. This allows more certainty in drawing of conclusions on temporal trends, which are not potentially influenced by single results of a country submitted for a single round. Therefore, the assessment of time trends for HCB of countries with repeated participation is part of the special chapter in Part IV (Malisch et al. 2023b).

5.2 African Group

HCB concentrations in all African countries in the whole period 2000–2019 were in the range of low background concentrations and varied between 1 and 5 µg/kg lipid (Fig. 19). A downward trend at the upper range of this background contamination is observed when results of Nigeria and Mauritius (in the 2008–2011 period) and Egypt (in the 2000–2003 period) are compared with results of these countries in the 2016–2019 period. In most other countries with repeated participation, the concentrations are quite stable at low concentrations of approximately 2–3 µg/kg lipid.

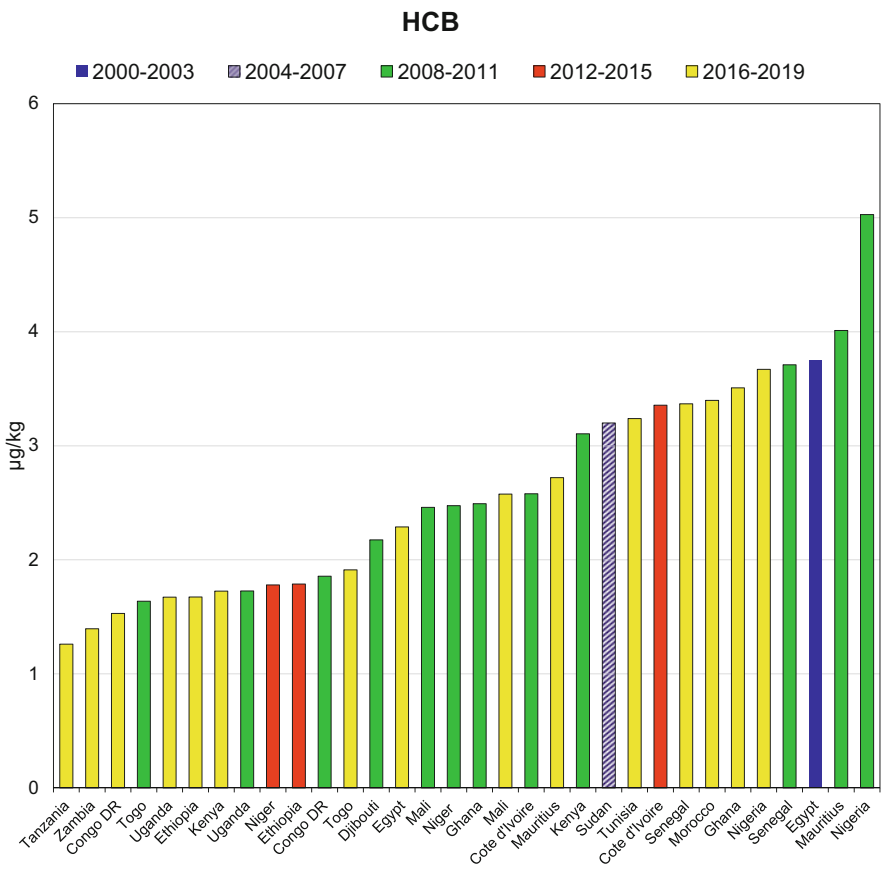

Fig. 19 Concentrations of HCB in human milk (µg/kg lipid) from countries in the African Group for the five periods from 2000 to 2019

5.3 Asia-Pacific Group

5.3.1 Asia Subgroup

Figure 20 illustrates the HCB results for the period 2000–2019 for the Asian countries of the Asia-Pacific Group. A wider range of concentrations was found with the lowest concentration in the sample from Cambodia, 2019 (2.5 µg/kg lipid) and the highest concentration in the one from Mongolia, 2018 (34 µg/kg lipid). Hong Kong SAR of China participated twice, with an increase from 2002 (19.3 µg/kg lipid as median of ten pooled samples [Hedley et al. 2010]) to the 2009 level of 25.8 µg HCB /kg lipid (median of four samples from different population subgroups). Two subgroups of 2009 with residents who had been living in Hong Kong for 10 years or more had comparable concentrations (20.5–21.0 µg/kg lipid) to 2002, whereas two subgroups of 2009 who had been living in Hong Kong for less than 10 years had concentrations of 30–35 µg/kg lipid.

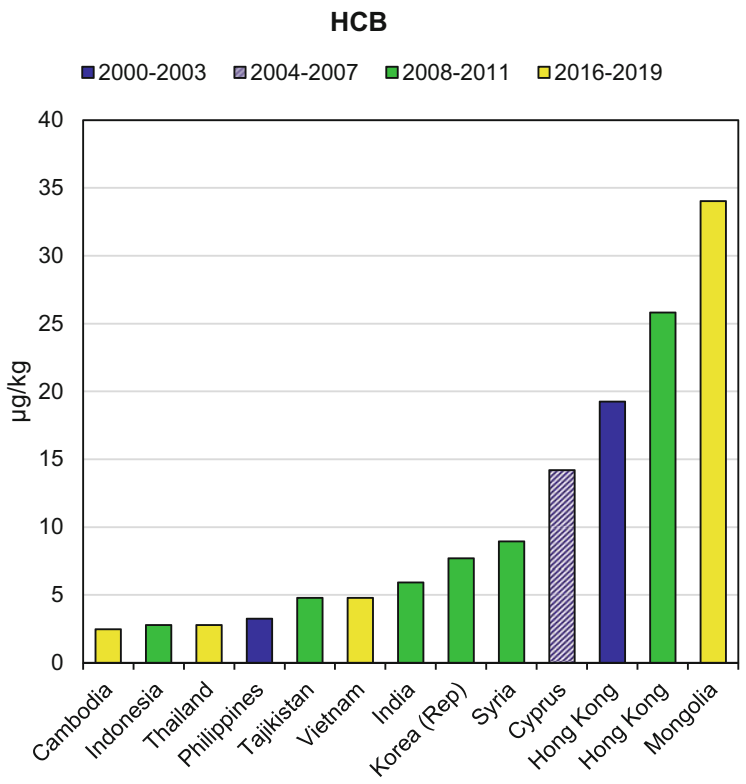

Fig. 20 Concentrations of HCB in human milk (µg/kg lipid) from countries in the Asia-Pacific Group/Asia subgroup for the five periods from 2000 to 2019

5.3.2 Pacific Islands Subgroup

Figure 21 illustrates the HCB results for the Pacific Islands countries of the Asia-Pacific Group. A downward trend is observed from the 2008–2011 period to the 2016–2019 period: Whereas the average background contamination in the 2008–2011 period was about 6 µg HCB /kg lipid, this dropped to about 3 µg/kg lipid in the 2016–2019 period. Four participations of Fiji between 2000 and 2019 showed quite stable background concentrations between about 3 and 5 µg/kg lipid.

5.4 Group of Latin American and Caribbean Countries (GRULAC)

Figure 22 illustrates the HCB results for Latin American and Caribbean countries. The results ranged from 1.0 µg/kg lipid (Haiti, 2015) to 14 µg/kg lipid (Uruguay, 2009). Three countries found at the upper end of this distribution all participated in the 2008–2011 period. From here, downward trends were observed in Uruguay from 2009 to 2019, in Mexico from 2011 to 2017, and in Chile from 2008 to 2011.

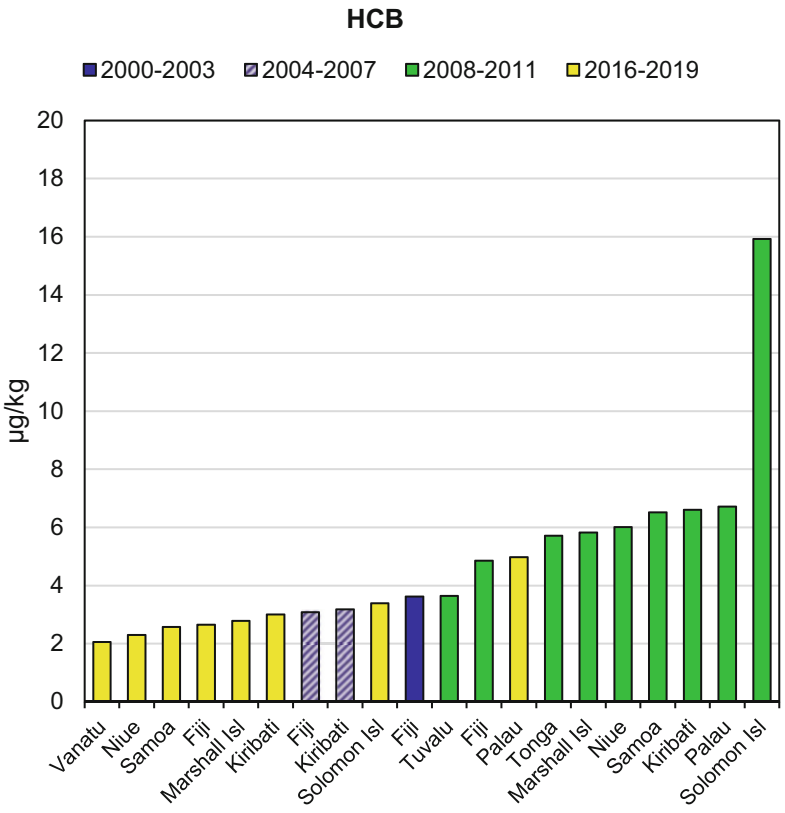

Fig. 21 Concentrations of HCB in human milk (µg/kg lipid) from countries in the Asia-Pacific Group/Pacific Islands subgroup for the five periods from 2000 to 2019

5.5 Eastern European Group

Figure 23 illustrates the HCB results for countries of the Eastern European Group. A wide range of concentrations was observed between the background contamination as found in Croatia, 2014 (7.6 µg/kg lipid) and Bulgaria, 2014 (7.7 µg/kg lipid), in comparison with 154 µg/kg found in Moldova, 2009. In Moldova, these concentrations decreased considerably to 46.7 µg HCB/kg lipid in 2015. The Czech Republic participated four times between 2000 and 2019; here, a continuous downtrend is found from 76 µg/kg lipid in 2001 to 12.5 µg/kg lipid in 2019. The intentional production in the Czech Republic ceased in 1968. The use as a pesticide was banned in Czechoslovakia in 1977, in Ukraine since 1997 (UNEP 2014).

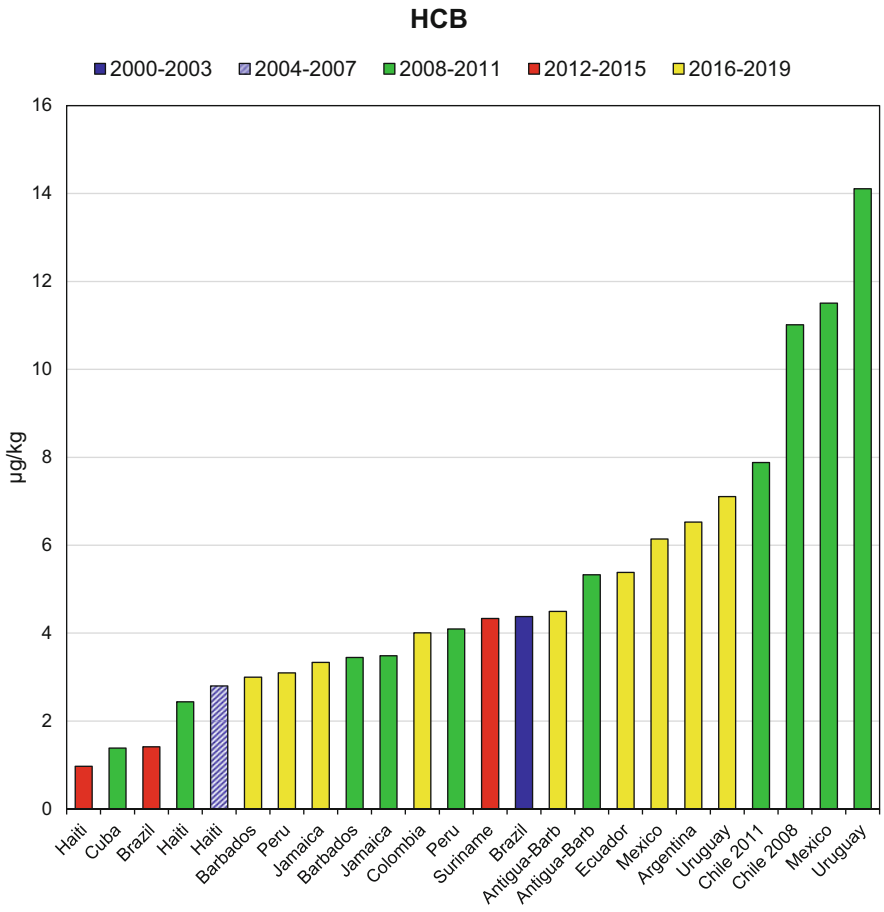

Fig. 22 Concentrations of HCB in human milk (µg/kg lipid) in Latin America and Caribbean countries for the five periods from 2000 to 2019

5.6 Western European and Others Group (WEOG)

Figure 24 illustrates the HCB results for countries of the Western European and Others Group. The concentrations ranged from 2.8 µg/kg lipid (Finland, 2007, median of three pooled samples) to 71.5 µg/kg lipid (Spain, 2002, median of three pooled samples). The highest concentrations were found in countries participating in the 2000–2003 period.

The gradual downward trend of the background contamination over the whole span of time between 2000 and 2019 can be illustrated by three participations of Ireland where background levels of HCB were at 13.5 µg/kg in 2002 and went down to 8.5 µg/kg lipid in 2019.

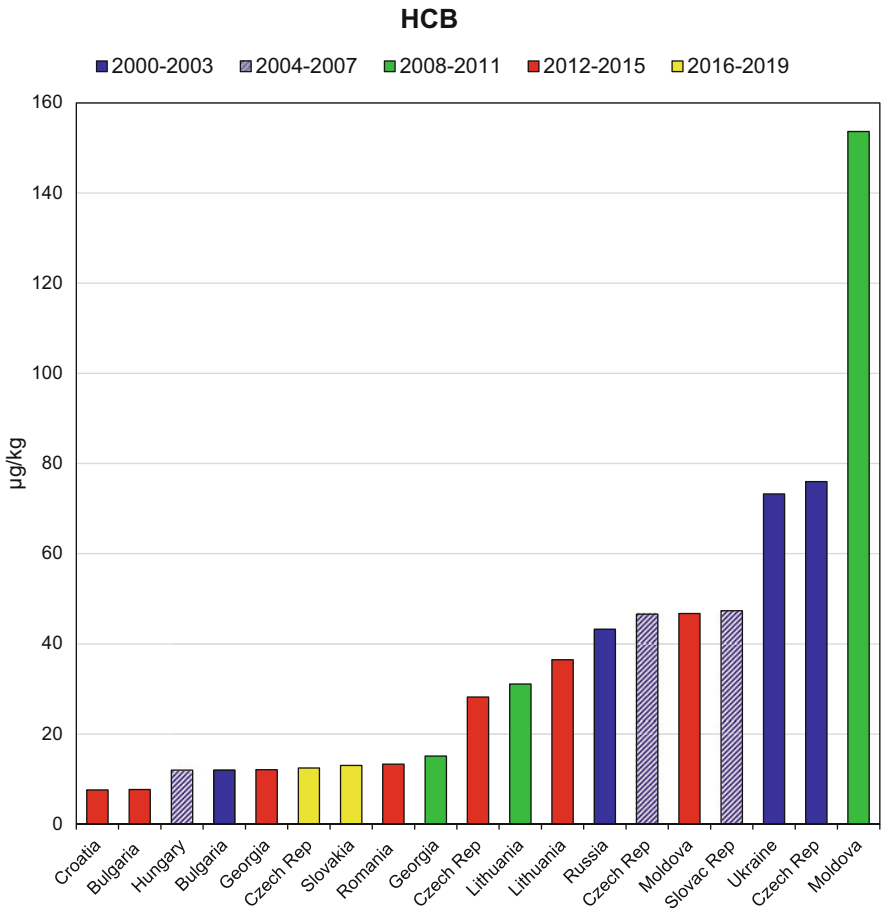

Fig. 23 Concentrations of HCB in human milk (µg/kg lipid) from countries in the Eastern European Group for the five periods from 2000 to 2019

6 Other Organochlorine Pesticides

6.1 Aldrin, Dieldrin

Aldrin and dieldrin belong to the group of the "12 initial POPs" under the Stockholm Convention. As the review on findings in humans in the introduction part shows, aldrin is rapidly metabolized to dieldrin, and therefore aldrin is not usually found in humans (Fürst 2023). Rather high dieldrin concentrations were reported in some countries (Fång et al. 2015).

Aldrin was not detected in any sample (<0.5 µg/kg lipid). Based on 134 country results, nearly 90% of the samples had dieldrin levels in a low background range

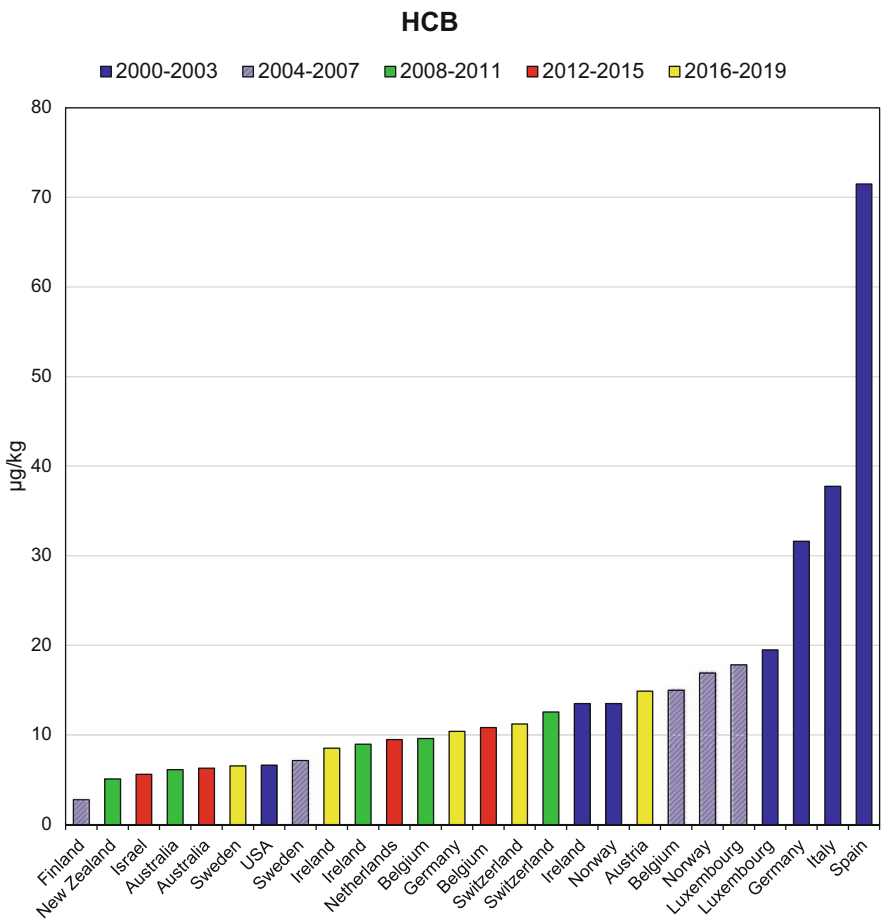

Fig. 24 Concentrations of HCB in human milk (µg/kg lipid) from countries in the Western European and Others Group for the five periods from 2000 to 2019

below 5 µg/kg lipid. Figure 25 illustrates the results for countries that are above 5 µg/kg lipid, with a maximum of 37.8 µg/kg lipid found in a sample from Tajikistan in 2009. A downward trend of the background levels for dieldrin from the 2000–2003 period to the 2016–2019 period was observed (Table 5).

6.2 Chlordane

Chlordane belongs also to the group of the "12 initial POPs" under the Stockholm Convention. Depending on the production process, technical chlordane can comprise more than 100 components. According to the residue definition for pesticides in food, the sum parameter "chlordane complex" comprises *cis*-chlordane (= "alpha-

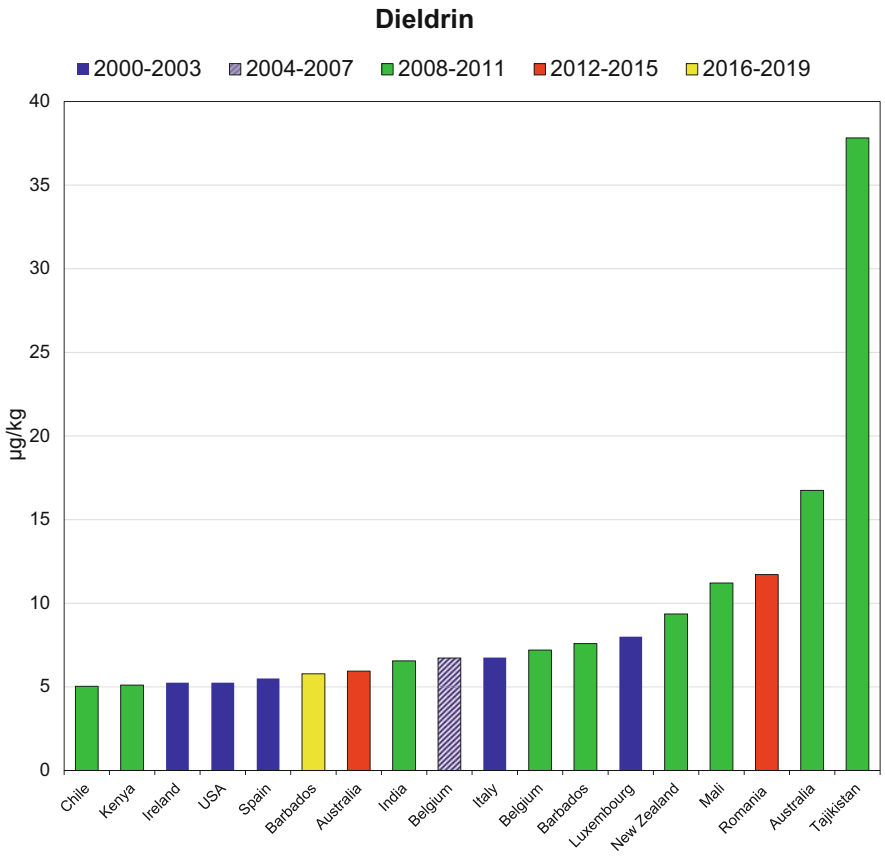

Fig. 25 Concentrations of dieldrin in human milk above 5 µg/kg lipid from countries in all UN regions in the five periods from 2000 to 2019

Table 5 Minimum, median, mean, and maximum concentrations of dieldrin in human milk (µg/kg lipid) from countries in all UN regions in the five periods between 2000 and 2019 with number of country results [*N*] (see Sect. 2.2)

	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019
N	16	13	45	17	43
Min	<0.5	<0.5	<0.5	<0.5	<0.5
Median	3.7	1.8	2.2	0.9	1.3
Mean	3.5	2.5	4.0	2.2	1.4
Max	8.0	6.7	37.8	11.7	5.8

chlordane") and *trans*-chlordane (= "gamma-chlordane") (both more relevant for food of plant origin) and the metabolite oxychlordane (relevant for food of animal origin). These were also the recommended analytes according to the Guidance on the Global Monitoring Plan (GMP) for POPs as of 2007 (UNEP 2007). Later, also *cis*-

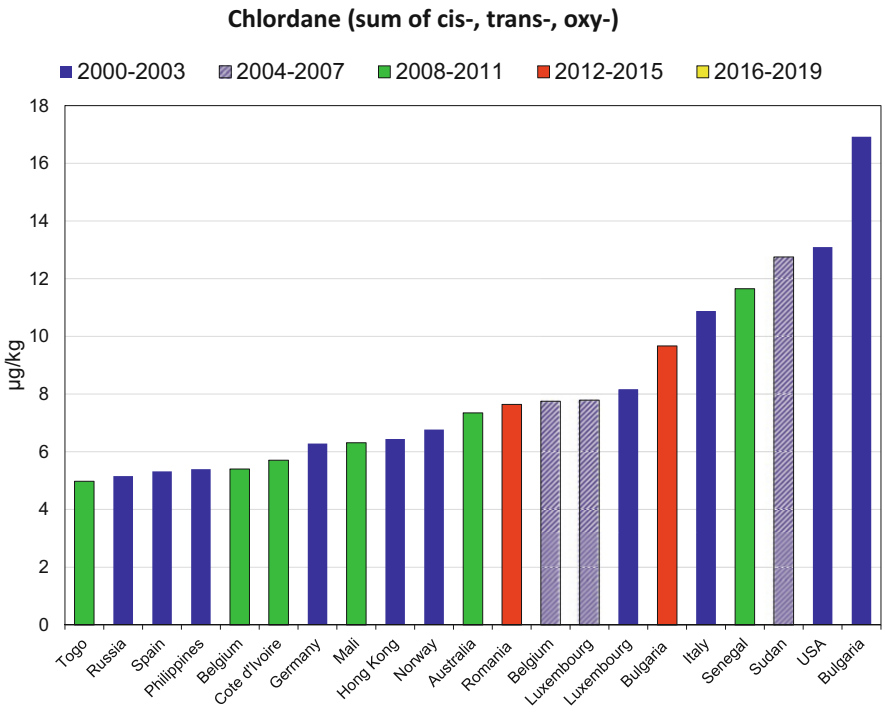

Fig. 26 Concentrations of chlordane in human milk above 5 µg chlordane complex/kg lipid from countries in all UN regions in the five periods from 2000 to 2019

and *trans*-nonachlor, which are impurities in chlordane production, were added to the list of recommended analytes for chlordane in the GMP guidance. Thus, the "chlordane group" comprises *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane (UNEP 2019). For definition of the sum parameters and correction factors for oxychlordane and nonachlor, see Sect. 2.3. For reported findings in humans, see the introduction part (Fürst 2023) and Fång et al. 2015.

Based on 134 country results for chlordane complex, determined as sum of *cis*- and *trans*-chlordane and oxychlordane, 23% of the samples had levels below the limit of quantification (0.5 µg/kg lipid), 61% in a low background range below 5 µg/kg lipid and 16% between 5 and 17 µg/kg lipid (Fig. 26). A downward trend was observed from the 2000–2003 period (median of 16 samples: 5.36 µg chlordane complex/kg; range 0.97–16.9) to the 2016–2019 period (median of 43 samples: 0.86 µg chlordane complex/kg; range 0.50–4.42).

In 42 of 44 samples, the nonachlor concentrations were below the background range of 5 µg/kg lipid, two samples were slightly above (Hong Kong SAR, 2002, 12.4 µg nonachlor /kg lipid; Barbados, 2018, 11.4 µg nonachlor /kg lipid).

6.3 Endrin

Endrin belongs to the group of the "12 initial POPs," as well. It is rapidly metabolized to endrin ketone. "Endrin complex" is the sum of endrin and endrin ketone. In contrast to reported findings (Fång et al. 2015), endrin and endrin ketone were not detected in any of 163 pooled samples of the periods 2000–2019 in which the limit of quantification was 0.5 µg/kg lipid.

6.4 Heptachlor

Heptachlor is also one of the "12 initial POPs." The review in the introduction part shows that it is also rapidly metabolized to heptachlor epoxide and that in humans cis-heptachlor epoxide can be found (Fürst 2023). Heptachlor complex is the sum of heptachlor, cis-heptachlor epoxide, and trans-heptachlor epoxide (for calculation of the sum parameter, see Sect. 2.3). As expected, in none of the 163 pooled samples heptachlor was found, in about half of them, heptachlor epoxide was below the limit of quantification (0.5 µg/kg lipid). In most cases of quantifiable residues, only cis-heptachlor epoxide was found with concentrations below 5 µg/kg lipid. Three samples were slightly above these low background levels, namely Luxembourg, 2002, 8.4 µg cis-heptachlor epoxide /kg lipid; Bulgaria, 2001, 12 µg cis-heptachlor epoxide /kg lipid; and Bulgaria, 2014, 14.1 µg cis-heptachlor epoxide /kg lipid.

6.5 Mirex

Mirex is another chemical that belongs to the group of the "12 initial POPs." It can be found in humans (Fång et al. 2015) but in about 80% of the samples of human milk of the WHO/UNEP-coordinated exposure studies, no mirex was detected (limit of quantification 0.5 µg/kg lipid). The remaining 20% of samples had concentrations in the range up to 3 µg/kg lipid with the exception of one sample that had a slightly higher level (Uruguay, 2009, 9.8 µg/kg lipid). However, Uruguay subsequently had a downward trend in 2019 to 2.9 µg/kg lipid.

6.6 Toxaphene

Toxaphene also belongs to the group of the "12 initial POPs." It is a complex mixture of chlorinated bornanes and chlorinated camphenes comprised of about 16,000 congeners/isomers. Marker compounds to be monitored are the congeners Parlar 26 (P26), Parlar 50 (P50), and Parlar 62 (P62) (UNEP 2019) as basis for the sum parameter toxaphene complex. This was calculated with application of the "lower bound approach" where only analytical results above the limit of quantification (0.5 µg/kg lipid) are used. Findings in humans were reported only in a few studies (Fång et al. 2015).

In 67% of the samples (134 country results), neither P26, P50 nor P62 was detected. In 32% of the samples, toxaphene was found in a range between 0.5 μg toxaphene complex/kg lipid and 6 μg toxaphene complex/kg lipid. Two older samples had higher concentrations, namely Norway, 2001, 10.0 μg toxaphene complex/kg lipid and Ukraine, 2001, 16.2 μg toxaphene complex/kg lipid.

6.7 Chlordecone

Chlordecone was listed in the Stockholm Convention in 2009. No studies were found reporting chlordecone concentrations in mother's milk (Fång et al. 2015). In none of 42 pooled samples submitted in the period 2016–2019 was chlordecone detected (limit of quantification 0.5 μg/kg lipid).

6.8 Endosulfan

Technical-grade endosulfan, which contains a number of related isomers, was listed in the Stockhom Convention in 2011. The sum parameter endosulfan complex comprises the analytes alpha-endosulfan, beta-endosulfan, and endosulfan sulfate (UNEP 2019; see Sect. 2.3). Only some scattered endosulfan mothers' milk data were found (Fång et al. 2015).

Of the 134 country samples, 98% did not have measurable residues above the limit of quantification (0.5 μg/kg lipid). Two samples had small amounts of about 1 μg endosulfan complex/kg lipid (Sudan, 2006; Brazil, 2012) and one sample had 6.3 μg endosulfan complex/kg lipid (Nigeria, 2009).

6.9 Pentachlorophenol (PCP), Pentachloroanisole (PCA)

Pentachlorophenol (PCP) was listed in the convention in 2015. PCP does not bioaccumulate, whereas its metabolite, pentachloroanisole (PCA), can be found after the use of PCP. Therefore, PCA is the recommended analyte for human milk (UNEP 2019).

As expected, none of 43 pooled samples of the 2016–2019 period had residues of PCP, and 41 of these samples had no residues of PCA (limit of quantification 0.5 μg/kg lipid). One sample had a trace of PCA (Fiji, 2019, 1.1 μg/kg lipid), while another sample had a high level of 33.3 μg/kg lipid (Vanuatu, 2018).

6.10 Dicofol

Dicofol was listed in the Stockholm Convention in 2019. Therefore, it was not included in the list of 23 POPs that were requested to be analyzed when the seventh round started in 2016. However, the analysis of dicofol was undertaken to have a

complete picture of all 30 POPs currently covered by the convention. Dicofol was detected in human breast milk from China, Korea, and Japan (Fujii et al. 2011).

In 40 samples which were submitted between 2017 and 2019, dicofol was not detected (limit of quantification 0.5 μg/kg lipid). Only one sample had a measurable level of dicofol at 3 μg/kg lipid (Ethiopia, 2019)

7 Organochlorine Industrial Chemicals

7.1 Pentachlorobenzene (PeCB)

Production and use of pentachlorobenzene (PeCB), e.g., formerly for various technical purposes, are prohibited by listing PeCB in the Stockhom Convention in 2009 in Annex A (Elimination). Furthermore, PeCB can also be formed as unintentional by-product during combustion and thermal and industrial processes. Therefore, PeCB is also listed in Annex C (Unintentional production) for minimization and, where feasible, ultimate elimination of unintentional releases (UNEP 2001).

In 90 of 99 pooled samples submitted during 2008 and 2019, no PeCB was found (limit of quantification 0.5 μg/kg lipid). In the nine remaining samples, low levels of between 0.5 and 1.2 μg/kg lipid were found.

7.2 Hexachlorobutadiene (HCBD)

Production and use of hexachlorobutadiene (HCBD), e.g., formerly for various technical purposes, are prohibited by listing HCBD in 2015 in Annex A (Elimination) of the Stockholm Convention. HCBD can also be formed as unintentional by-product and was therefore listed in 2017 also in Annex C (Unintentional production) (UNEP 2001).

HCBD was not detected above the limit of quantification (0.5 μg/kg lipid) in any of the 43 pooled samples submitted in the period 2016–2019.

8 Summary

Between 2000 and 2019, five rounds of human milk studies on persistent organic pollutants (POPs) were coordinated by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) with 82 countries from all the five United Nations regions. The intent of these studies was to evaluate the effectiveness of the Stockholm Convention on POPs in eliminating or reducing emissions of selected POPs. Countries submitted composite samples prepared by pooling many individual samples. These pooled samples are considered representative of the national average of the analytes of interest at the time of sampling. The concentrations of 30 POPs presently listed in the convention were determined, among them the 18 organochlorine pesticides and industrial contaminants reported

in this article, including the parent molecules, certain metabolites, degradation products and/or by-products during manufacture if recommended by the Guidance Document on the Global Monitoring Plan. If a country provided two or more pooled samples for a certain round, the median of results is used to represent the country. Up to 134 country results were available for this evaluation. Note that the results presented here are based on UN regions and are not intended for ranking of individual countries.

For dichlorodiphenyltrichloroethane (**DDT**), huge differences were found, with a minimum of 17 μg DDT complex/kg lipid found in 2019 in a country from the African Group and 23,500 μg DDT complex/kg lipid found in 2012 in another country from the African Group (median of 134 country results: 255 μg DDT complex/kg lipid). The summarizing parameter (DDT complex) comprises the *p,p′*- and *o,p′*-isomers of DDT and their metabolites dichlorodiphenyldichloroethylene (*p,p′*-DDE; *o,p′*-DDE) and dichlorodiphenyldichloroethane (*p,p′*-DDD; *o,p′*-DDD), respectively. Because *p,p′*-DDT is extensively metabolized, its transformation product *p,p′*-DDE contributes on average about 95% to the sum parameter "DDT complex." Consequently, in most human milk samples, *p,p′*-DDT only contributes about 5% to DDT complex. However, in cases of a more recent use and contamination, considerably higher contributions of p,p′-DDT can be found. As an example, 46% *p,p′*-DDT was found in a sample of 2012 from a country in the African Group probably due to public health use of DDT to combat mosquitos for malaria control, which is permitted under the Stockholm Convention with some constraints.

As a general estimation of time trends, the median of the DDT complex concentrations of all samples shows a decrease of 72% from the 2000–2003 period (median for 16 countries: 445 μg DDT complex/kg lipid) to the 2016–2019 period (median for 43 countries: 125 μg DDT complex/kg lipid). The downward trend was found in all UN regions although considerable differences between groups and rounds were noted. The assessment of time trends based only on country-specific results of countries with repeated participation allows more certainty in drawing of conclusions, and therefore is optimal for the evaluation of the effectiveness under of Article 16 of the convention. This evaluation is published separately in Part IV of this compendium.

Due to metabolization of hexachlorocyclohexanes (**HCH**) in humans, the concentrations of alpha-HCH and gamma-HCH were, in most human milk samples, below the limit of quantification (<0.5 μg/kg lipid) with a median of quantifiable residues of about 1 μg/kg lipid for alpha-HCH and gamma-HCH and with maxima of 10.5 μg/kg for alpha-HCH and 16 μg/kg for gamma-HCH. In most cases when the sum of HCH isomer concentrations was above 10 μg/kg lipid, about 95–100% of this is due to beta-HCH, which is the HCH isomer that mainly bioaccumulates in humans. The ranges of beta-HCH found in the five studies over 20 years varied considerably among UN regions. Residues below the limit of quantification (<0.5 μg/kg lipid) were found in few countries. The lowest beta-HCH concentrations were found in Pacific Islands countries (range: 0.5–6.5 μg beta-HCH /kg lipid). With an overall median of 5.9 μg beta-HCH/kg lipid, the 134 results for all countries were in a similar range. The maximum of 1020 μg beta-HCH/kg

lipid was found in 2002 in an Asian country of the Asia-Pacific Group, but the levels have significantly fallen since then.

Based on the median of the beta-HCH concentrations of all samples, a decrease of 91% was found from the 2000–2003 period (median for 16 countries: 25.3 µg beta-HCH /kg lipid) to the 2016–2019 period (median for 43 countries: 2.4 µg beta-HCH / kg lipid). However, in the three rounds in-between, considerable variations in results from single submissions by countries were observed. Therefore, a time trend for beta-HCH is also included in the separate assessment of time trends based only on results from countries with repeated participation in the studies presented separately in Part IV.

In comparison with DDT and HCH, the maximum levels and ranges for hexachlorobenzene (**HCB**) were much lower, with a minimum of about 1–2 µg/kg lipid found in some countries and maximum of 154 µg HCB /kg lipid found in 2009 in a country of the Eastern European Group. With median levels between 12.8 µg/kg lipid in the 2016–2019 period and 58.3 µg/kg lipid in 2000–2003 period, the Eastern European Group had the highest median concentrations for five periods. The median of 134 country results was 5.1 µg/kg lipid. All samples from the African Group were in the low background range below 5 µg/kg lipid. This was also true for many countries from the Asia-Pacific Group and the Latin American and Caribbean Group.

In general, concentrations of HCB show a downwards trend: The median of all samples decreases from the 2000–2003 period (16 countries, 16.4 µg/kg lipid) to the 2016–2019 period (43 countries, 3.3 µg/kg lipid) by 80%. In the Eastern European Group, the median of the HCB concentrations drops from the 2000–2003 period (58 µg/kg lipid) to the 2012–2015 period and then plateaus at about 13 µg/kg lipid. In countries of the Western European and Others Group, a downward trend is observed from the 2000–2003 period (about 20 µg/kg lipid) to the 2008–2011 period and then levels out to around 10 µg/kg lipid. In other groups, the background contamination levels out at a concentration of up to 5 µg/kg lipid.

The following organochlorine pesticides and contaminants and their metabolites were mostly found in ranges of low background contamination; some were below the limit of quantification for the analytes of interest.

Aldrin is rapidly metabolized to **dieldrin** and therefore, aldrin was not detected in any sample (limit of quantification 0.5 µg/kg lipid). About 90% of the samples had dieldrin levels in a low background range below 5 µg/kg lipid. Four samples had dieldrin concentrations above 10 µg/kg with a maximum of 37.8 µg/kg lipid. A downward trend of the background levels for dieldrin from the 2000–2003 period to the 2016–2019 period is observed.

Recommended analytes for **chlordane** (chlordane complex) included initially *cis*-chlordane (or "alpha-chlordane") and *trans*-chlordane (or "gamma-chlordane") and the metabolite oxychlordane. Later, also *cis*- and *trans*-nonachlor were added (chlordane group), which can be found as impurities in chlordane. Based on the analysis for chlordane complex, determined as sum of *cis*- and *trans*-chlordane and oxychlordane, the results for 134 country samples show that 23% of the samples had levels below the limit of quantification (0.5 µg/kg lipid), 61% in a low background

range below 5 μg chlordane complex/kg lipid, and 16% between 5 and 17 μg chlordane complex/kg lipid. A downward trend is observed from the 2000–2003 period to the 2016–2019 period. In 42 of 44 samples, the nonachlor concentrations were below 5 μg nonachlor /kg lipid, with two samples up to 12.4 μg nonachlor /kg lipid.

Endrin and its metabolite endrin ketone were not detected in any sample of the 2000–2019 period (limit of quantification 0.5 μg/kg lipid).

Heptachlor is rapidly metabolized in the body to heptachlor epoxide. In about half of the 134 country results, heptachlor epoxide was below the limit of quantification (0.5 μg/kg lipid). In most cases of quantifiable residues, cis-heptachlor epoxide was found with concentrations below 5 μg/kg lipid. Three samples were slightly above these low background levels with a maximum of 14.1 μg cis-heptachlor epoxide /kg lipid.

In about 80% of the samples, no **mirex** was detected (limit of quantification 0.5 μg/kg lipid); most of the remaining 20% of samples had concentrations in the range up to 3 μg/kg lipid. Only one sample had slightly higher levels (9.8 μg/kg lipid).

Monitoring for **toxaphene** (toxaphene complex) is based on the congeners Parlar 26 (P26), Parlar 50 (P50), and Parlar 62 (P62). In 67% of the samples, neither P26, P50 nor P62 was detected (limit of quantification 0.5 μg/kg lipid). In 32% of the samples, toxaphene was found in a range between 0.5 and 6 μg toxaphene complex/kg lipid, calculated as sum of P26, P50, and P62. Two samples of 2001 had slightly higher concentrations with a maximum of 16.2 μg toxaphene complex/kg lipid.

Chlordecone was not detected in any of 42 pooled samples submitted in the period 2016–2019 (limit of quantification 0.5 μg/kg lipid).

Analysis for **endosulfan** (endosulfan complex) comprises the determination of alpha-endosulfan, beta-endosulfan, and endosulfan sulfate. About 98% of all samples did not have quantifiable residues of these recommended analytes (limit of quantification 0.5 μg/kg lipid). Two samples had small traces of about 1 μg endosulfan complex/kg, while one sample had 6.3 μg endosulfan complex/kg.

Pentachlorophenol (PCP) does not bioaccumulate in humans; however, the metabolite **pentachloroanisole (PCA)** can be found after use of PCP. While none of 43 pooled samples of the 2016–2019 period had residues of PCP, only 2 of these samples had residues of PCA (limit of quantification 0.5 μg/kg lipid). One sample had traces of PCA (1.1 μg/kg lipid) and another sample had 33.3 μg/kg lipid.

Dicofol was not detected in 40 out of 41 samples, which were submitted between 2017 and 2019 (limit of quantification 0.5 μg/kg lipid). In one sample from the African group, a level of 3 μg/kg lipid was found.

In 90 of 99 pooled samples of the periods covering 2008–2019, no **pentachlorobenzene** (PeCB) was found (limit of quantification 0.5 μg/kg lipid); in nine samples, traces between 0.5 and 1.2 μg/kg lipid were found.

No residues of **hexachlorobutadiene** (HCBD) were detected (limit of quantification 0.5 μg/kg lipid) in any of the 43 pooled samples, submitted in the period 2016–2019.

In conclusion, these results demonstrate that for many of the POPs covered by this article, the levels of POPs in human milk have generally fallen over the last 20 years with higher levels seen only in sporadic cases. For other POPs, the results were low or below the limit of quantification. Most significantly, human milk samples are a key matrix for the effectiveness evaluation of the Stockholm Convention; their analysis as pooled samples considered to represent the average levels of POPs for a country at the time of sampling has been shown to be a very cost-effective approach for monitoring levels POPs on a global level, allowing to draw conclusions (i) on the relative importance of particular POPs in regions, (ii) on the priority of the individual POPs among the whole list of 30 compounds as covered under the Stockholm Convention as of 2019, and (iii) on temporal trends.

Acknowledgments This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region," funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk studies, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure studies for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the WHO/UNEP Reference Laboratories, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP, and the Reference Laboratories was an important aspect for the successful organization of these studies on a global level.

Hae Jung Yoon, Seongsoo Park, and Philippe Verger (WHO Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

We thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam, and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao, and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects during her time at the UNEP Economy Division, Chemicals and Health Branch.

We also thank the team at CVUA Freiburg for the performance of the analyses of the POPs presented in this publication.

Disclaimer The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions, policy, or views of the World Health Organization and the United Nations Environment Programme.

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WHO- and UNEP-Coordinated Exposure Studies 2000–2019: Findings of Polybrominated Substances (PBDE, HBCDD, PBB 153, PBDD/PBDF)

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Abstract

The concentrations of a number of polybrominated substances were determined in pooled human milk samples collected from up to 80 countries from all the United Nations Regional Groups. The samples were taken from one or more of the five exposure studies on persistent organic pollutants coordinated by the

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_9

World Health Organization and the United Nations Environment Programme between 2000 and 2019.

Large differences in levels were found for polybrominated diphenyl ethers (PBDE). The concentration of the sum of 6 PBDE congeners ($\sum PBDE_6$: BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183) of 135 pooled samples from 80 countries was in the range between 0.3 and 352 ng/g lipid (median 1.6 ng/g lipid). The highest concentrations were detected in the Western European and Others Group (including Australia, Israel, New Zealand, and the USA) in 2003. Time trends were assessed for 36 countries with repeated participation. Rates of decrease tend to fluctuate at near background levels; but at higher levels, a decreasing trend was observed for nearly all countries. Deca-BDE (BDE-209) contributed on average about 13% to $\sum PBDE_7$ (sum of $\sum PBDE_6$ plus BDE-209). The contribution of the octa-brominated diphenyl ether (octa-BDE) BDE-197 and nona-BDEs-206, -207, and -208 to the sum of 25 PBDE was in the range of the six recommended analytes and BDE-209. Therefore, their addition to the list of recommended analytes should be considered.

The α-HBCDD levels of 102 pooled samples from 72 countries collected between 2006 and 2019 ranged between <0.1 and 15 ng/g lipid (median: 0.5 ng/g lipid). β-HBCDD and γ-HBCDD were in nearly all samples below the limit of quantification (LOQ for 90% of the samples: <0.1 ng/g lipid) or around the LOQ (max: 0.8 ng/g lipid). Thus, it can be concluded that α-HBCD is the predominant stereoisomer in human milk.

Hexabromobiphenyl (PBB 153) was below the limit of quantification (0.5 ng/g lipid) in 106 of 110 pooled samples from 69 countries. In four samples, low concentrations of between 1.0 and 1.7 ng/g lipid were found.

In addition to these chemicals listed by the Stockholm Convention on Persistent Organic Pollutants, in 38 pooled samples from 28 countries concentrations of polybrominated dibenzodioxins and -furans (PBDD/PBDF) were determined to assess their contribution to the overall sum of WHO_{2005} toxic equivalents (TEQ) with polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). PBDD and PBDF provided on average about 10% to the overall TEQ calculated as sum of WHO-PCDD/PCDF-TEQ and WHO-PBDD/PBDF-TEQ, when assuming the same toxic equivalency factors for brominated congeners as assigned to their chlorinated analogs. No correlations between PCDD/PCDF and PBDD/PBDF, or PBDD/PBDF and PBDE were found.

Keywords

Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · Polybrominated diphenyl ether (PBDE) · Hexabromocyclododecane (HBCD or HBCDD) · Hexabromobiphenyl (HBB) · Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/PBDF) · Global WHO/UNEP human milk studies · UN regional groups · Time trends

1 Introduction

A number of organobromine compounds with an inhibitory effect on combustion processes are used as flame retardants (brominated flame retardants [BFR]). Among them are the polybrominated diphenyl ethers (PBDE), hexabromocyclododecanes (usual abbreviations: HBCD or HBCDD; in this article used: HBCDD), and hexabromobiphenyl (HBB). As chemically and physiologically stable substances, they persist in the environmental and biological systems. Due to their lipophilic properties and often slow metabolization, biomagnification in the food chain and bioaccumulation in fatty tissues of animals and humans occurs readily. For these reasons, these compounds are formally classified as persistent organic pollutants (POPs) (UNEP 2017).

To protect human health and the environment, the Stockholm Convention on POPs has identified selected POPs to reduce or eliminate their release into the environment (UNEP 2001). In 2009, hexabromobiphenyl and tetra-, penta-, hexa-, and heptabromodiphenyl ethers were listed under the Convention, followed in 2013 by HBCDD and in 2017 by decabromodiphenyl ether (Deca-BDE) (UNEP 2020). As a result, these POPs are now subject to Article 16, which requires that they be monitored to evaluate the effectiveness of the Convention. The analysis of those POPs in human milk has been recommended as one of the core matrices within the framework of the Global Monitoring Plan on POPs (GMP) (UNEP 2019).

Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/PBDF) can be found in a wide variety of abiotic and biological matrices. Sources for environmental release include commercial mixtures of BFR, in which PBDD and PBDF may occur as impurities. In addition, de novo formation of these compounds occurs during combustion processes in which bromine is present. During these processes, significant amounts of PBDD and PBDF can be formed, including those congeners that are analogs of the most toxic chlorinated dioxins and dibenzofurans (PCDD/PCDF) in terms of their halogen substitution pattern. Because of their similarity in biological and toxicological properties with chlorinated analogs, the World Health Organization (WHO) has established interim values for Toxicity Equivalency Factors (TEF). Due to the similarity in toxic properties between chlorinated and brominated dioxins and dibenzofurans both groups of compounds should be combined in this TEF concept (van den Berg et al. 2013), until sufficient data on their toxicity is available to propose more specific values. In contrast to PCDD and PCDF, PBDD and PBDF are not listed by the Stockholm Convention on POPs. However, their determination is of scientific interest to assess the contribution of brominated dioxins and dibenzofurans to toxic equivalents (TEQ).

Human milk surveys are reviewed in this compendium from various aspects. The general introduction (Part I) gives a review of human milk surveys on POPs (Fürst 2023), an overview of the WHO and United Nations Environment Programme (UNEP) coordinated exposure studies performed between 1987 and 2019 (Malisch et al. 2023a) and a review of the Stockholm Convention on POPs and its implementation by regional and global monitoring reports (Šebková 2023). In Part II, specific analytical aspects are presented, including methods used for determination of PBDE,

HBCDD, HBB, and PBDD/PBDF (Schächtele et al. 2023). In various articles of Part III, the analytical results for chlorinated and brominated POPs between 2000 and 2019 are reported and discussed. Part IV includes an assessment of time trends based on these WHO/UNEP-coordinated human milk surveys and is finalized with a review of possible health risks and benefits for the breastfed infant deriving from dioxin-like compounds. Conclusions and key messages are presented in Part V.

A former publication presented the results of the third round of WHO-coordinated exposure studies (2000–2003) and showed that PBDE levels in human milk from the USA were a factor of 35–500 higher than those in other countries. The correlation between PBDE and PBDD/PBDF was assessed (Kotz et al. 2005; Kotz 2006). A comprehensive report for the 6th Conference of the Parties to the Stockholm Convention in 2013 provided an overview on all samples of the three studies spanning the period 2000–2012. It revealed large global differences among various POPs, including PBDE (UNEP 2013).

A global overview on the spatial and temporal trends of Stockholm Convention POPs in breast milk reviews scientific publications between 1995 and 2011. It includes summaries of data for concentrations of PBDE and HBCDD (Fång et al. 2015). The regional and global monitoring reports for the GMP assess datasets in the core media—ambient air, human tissues (human breast milk or blood), and water for hydrophilic POPs, but also other media such as soil, biota, plants are used to support interpretation of observed levels and their trends (UNEP 2022).

All substance-specific data are deposited at the Global Monitoring Plan Data Warehouse (GMP DWH), which can be publicly accessed. This serves as the source of information for the regional and global reports of the GMP and for the evaluation of the effectiveness of the convention to eliminate or reduce emissions of selected POPs (GMP DWH 2020).

In this article, the results for up to 135 pooled samples from up to 80 countries collected between 2000 and 2019 are discussed from various perspectives in the following sections, namely: Sect. 2 General aspects; Sect. 3 Polybrominated diphenyl ethers (PBDE); Sect. 4 Hexabromocyclododecanes (HBCDD); Sect. 5 Hexabromobiphenyl (PBB 153); and Sect. 6 Polybrominated dibenzodioxins and -furans (PBDD/PBDF).

2 General Aspects

2.1 Link to the General Introduction (Countries, UN Regions, Protocol)

An overview of the scope, protocols for collection of samples, expansion of analytes of interest over time by the Stockholm Convention, participation of countries with classification in UN regional groups and temporal differentiation are given in the general introduction in Part I (Malisch et al. 2023a). In brief, the collection of a number of individual samples and preparation of representative pooled samples in all rounds were supervised by a national coordinator in each country following the

WHO/UNEP-standardized protocols. Equal aliquots of the individual samples were combined to give a composite sample, which is considered to represent the average levels of POPs for a country or a subpopulation at the time of sampling. The pooled samples were sent to the WHO/UNEP Reference Laboratories for analysis.

In accordance with the implementation of the GMP, parties report through one of the five United Nations regional organization groups. Therefore, countries are classified according to one of these five UN geopolitical groups: (1) African Group, (2) Asia-Pacific Group, (3) Eastern European Group, (4) Group of Latin American and Caribbean Countries (GRULAC), and (5) Western European and Others Group (WEOG). Note that Australia, Israel, New Zealand, and the USA (being informally a member) are included as "Others" in WEOG countries (for participating countries and regional distribution, see Section 6 in Malisch et al. 2023a). It should, furthermore, be noted that these results are not intended to be used for the ranking of individual countries or regions.

2.2 Analysis of Polybrominated Substances

The analytical methods for determination of polybrominated compounds and their validation are presented in Part II (Schächtele et al. 2023). All concentrations are reported on a lipid basis.

2.3 Number of Samples and Aggregation of Data

Due to the particular scope at the beginning of a study with regard to the expansion of analytes of interest over time, a total of 135 pooled samples from 80 countries submitted between 2000 and 2019 were analyzed for **PBDE**. In the 2000–2003 survey, countries were encouraged to submit at least two pooled samples, whereas in the following rounds, usually one pooled sample was submitted per country. If two or more pooled samples were available for a country in a certain round, the median of the individual results is used for aggregation purposes. This yielded eight median results that are used to represent the country for that round (Australia, 2013; Belgium, 2002; Fiji; 2006; Finland, 2007; Germany, 2002 and 2019; Ireland, 2001; USA, 2003). Thus, for 80 countries (with 36 countries participating repeatedly between 2000 and 2019), 124 "country results" are available for the "one country—one result for a certain period" approach. However, for the time trend analysis, data were not aggregated, and values of all 135 pooled samples were used for time trend analysis. As **BDE-209** was not listed until 2017 in the annexes to the Stockholm Convention, only 40 samples from 39 countries submitted in the 2016–2019 period were analyzed for this compound.

In addition, 102 pooled samples from 72 countries collected between 2006 and 2019 were analyzed for the three stereoisomers **α-, β-, and γ-HBCCD,** 110 pooled samples from 69 countries for **hexabromobiphenyl (PBB 153)** and furthermore for scientific reasons 38 samples from 28 countries from the period 2001 and 2009 for **PBDD/PBDF** (not listed by the Stockholm Convention on POPs).

The detailed data for all these samples are included in the POPs Global Monitoring Plan Data Warehouse and can be publicly retrieved (GMP DWH 2020).

2.4 Methods of Statistical Data Treatment for Time Trends of PBDE in Countries with Repeated Participation

For the time trend analysis of PBDE in countries with repeated participation (Sect. 3.2), data were not aggregated. Instead, values of all submitted pooled samples were used for these trend analyses. For methods of statistical data treatment, see the chapter on time trends for PCB, PCDD, and PCDF in Part IV (Malisch et al. 2023c). In brief, the GMP guidance document recommends to apply simple linear regression or the Theil-Sen estimator for power analysis of statistical trends (UNEP 2019). Therefore, the non-parametric linear Theil-Sen trend estimator (Sen 1968; Theil 1992) was used for derivation of the exponential trends as expected after stop of production and application of chemicals (Sharma et al. 2021). This approach is in line with various elimination studies deriving half-lives for PBDE in rodents and humans. A review shows a wide range of elimination half-lives depending on the number of bromine substituents and congener, including a model for serum half-lives of PBDE with 7–10 bromine substituents after exposure of rubber workers and electronic dismantlers (EFSA 2011; Thuresson et al. 2006).

R package "Median-based Linear Models (mblm)" (Komsta 2013) was used for this regression. To prevent biased results in case the kinetics is different to the 1st order exponential decrease, an additional assessment was done considering a case with a small but persistent exposition to the compounds using the PORT method for non-linear regression (Fox 1984). For each trend, its statistical significance was estimated, showing on 95% confidence level whether the trend is not caused by random variance in the data and R^2 value was used as an indicative measure of a goodness of fit. In the assessment of temporal trends, improper results can be induced by use of samples from different countries in different times ("Simpson's paradox" as a statistical phenomenon in which a trend appears in certain groups of data but disappears or reverses when the groups are combined, Simpson 1951). To prevent this, two additional analyses were made: (1) computing of annual decrease rates separately for two decades and comparison of slopes of trends using z-scores (Fisher 1915); (2) deriving the regional or global trend as a median of trends in countries within the region or globally ("median method").

2.5 Background Concentrations Versus High Concentrations After Exposure

Background concentrations are defined as that portion of the measured human milk levels that is found in the absence of specific sources and therefore not attributable to a known exposure such as use of the chemical of interest or emissions within the study area. In contrast to findings of high concentrations caused by use or emissions

of a chemical, considerably lower concentrations might be found for many POPs when its use or emissions have ceased for a longer period of time. These levels are then described as "background levels."

Reduction rates should be seen in context with the concentration range (levels above or in the range of background contamination): If high levels are found, sources might be detected, which could be reduced or eliminated and would then result in decreasing trends. However, at low background levels, other factors, e.g., contamination of feed and food by air via long-range transport or from subsequent bioaccumulation, cannot be influenced locally. As a possible consequence, concentrations might level out or some fluctuation of calculated decrease rates might be observed.

It should be noted that the term "background level" does not imply per se any level of safety. With respect to potential adverse health effects, subsequent risk assessments need to consider a variety of factors, including the toxicity of the chemical of interest and the concentration found. For human milk, obviously the potential adverse effects have to be balanced against positive health aspects for (breastfed) infant. Such a risk–benefit evaluation of breastfeeding for dioxin-like compounds is included in the assessment chapters in Part IV (van den Berg et al. 2023).

3 Polybrominated Diphenyl Ethers (PBDE)

There are theoretically 209 congeners of polybrominated diphenyl ethers. The most important technical products were composed of a mixture of congeners and were named according to their average bromine content. To avoid confusion, EFSA proposed to use names in capital letters when referring to technical mixtures (e.g., PentaBDE), whereas lowercase letters were used to refer to the homologues itself (e.g., pentaBDE). The three commercial mixtures were: PentaBDE, Octa-BDE, and Deca-BDE (EFSA 2011). These have been produced in the past in large volumes (e.g., volume [metric tons] estimates in 2001: 56,100 t Deca-BDE, 3790 t Octa-BDE, 7500 t Penta-BDE (Birnbaum and Staskal 2004)). PBDE were widely used in polymers and textiles, construction materials, furniture, and electric and electronic equipment. As additive flame retardants, they are not chemically bound to the polymers and can therefore leach into the environment.

The commercial products contained mainly tetra- and pentabromodiphenyl ethers (Penta-BDE), hexa- to nonabromodiphenyl ethers (Octa-BDE) or decabromodiphenyl ether (Deca-BDE) (De Wit 2002; Alaee et al. 2003; La Guardia et al. 2006; EFSA 2011). Tetra-BDE and penta-BDE (as main components of commercial Penta-BDE products [UNEP 2009a]) and hexa-BDE and hepta-BDE (as main components of commercial Octa-BDE products [UNEP 2009b]) were listed under Annex A (for elimination) in the Stockholm Convention in 2009, deca-BDE (as main component of the commercial Deca-BDE mixture) in 2017 also in Annex A (UNEP 2020).

Table 1 Number of theoretically possible PBDE congeners and recommended analytes (UNEP 2019)

PBDE	Listed	No of theoretically possible congeners	Recommended analytes
Tetra- and pentabromodiphenyl ether	2009	Two homolog groups: 42 tetrabrominated isomers; 46 pentabrominated isomers	BDE-47, -99, Optional: BDE-100
Hexa- and heptabromodiphenyl ether	2009	Two homolog groups: 42 hexabrominated isomers; 24 hepta-brominated isomers	BDE-153, -154, -175/183 (co-eluting)
Decabromodiphenyl ether	2017	Single compound	BDE-209

Although there is a large number of theoretically possible isomers as parent compounds, the Guidance Document on the Global Monitoring Plan for POPs recommends only six prevalent analytes (comprising seven congeners) from tetra-BDE to hepta-BDE (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-175/183 [co-eluting]) for commercial Penta-BDE and Octa-BDE products, furthermore BDE-209 for Deca-BDE (UNEP 2019) (Table 1).

However, questions were raised whether BDE-175 is an important enough component of commercial octabromodiphenyl ether mixtures to be listed in Annex A of the Stockholm Convention. Since BDE-175 and BDE-183 co-elute on common HRGC columns, the presence of BDE-175 as an important component in technical Octa-BDE mixtures has not been illustrated. The successful HRGC/LRMS separation of a 1:1 mixture of BDE-175 and BDE-183, as well as ^{1}H NMR analysis of technical material, has allowed to confirm that this congener is not present in technical products (Great Lakes DE-79™ and Bromkal 79-8DE™) in quantifiable amounts (Konstantinov et al. 2011). Therefore, the "Guidance Document on the Determination of Organobromine Contaminants for analytical parameters in food and feed" recommends 9 PBDE congeners as analytes of interest: seven congeners covered by the GMP Guidance Document (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209) and in addition BDE-28 and BDE-49 (EURL for Halogenated POPs in Feed and Food 2022).

As summarizing parameter for human milk samples, the sum of the six recommended PBDE analytes ($\sum$ $PBDE_6$ as sum of BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183, however without consideration of BDE-175) was reported by CVUA Freiburg until in 2017, when BDE-209 was added. The Second Global Monitoring Report uses two kinds of sum parameters for PBDE concentrations in human tissues: (1) for human milk, the sum of the six PBDE analytes is given as "sum 7 PBDEs"; (2) for blood of mothers, the sum of four congeners (sum of BDE-47, BDE-99, BDE-100, and BDE-153) is given (UNEP 2017).

All congeners recommended to be analyzed bioaccumulate in humans (Fürst 2023).

3.1 Sum of Six PBDE Congeners (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183)

Table 2 compiles the most important statistical data (number of samples, minimum, median and maximum) of the sum of the six recommended PBDE congeners in human milk (expressed as ng $\sum$ $PBDE_6$/g lipid) for the 124 country results (median as one result per country where data for two or more pooled samples for one period are available, see Sect. 2.3) for 80 countries from the five UN Regional Groups. A considerable variation of the PBDE concentrations in human milk in the five periods between 2000 and 2019 was observed, depending on the inclusion of individual countries in a certain round. Of all samples, 80% were in a range below 5 ng $\sum$ $PBDE_6$/g lipid, including all samples from Africa and the Eastern European Group. The highest concentration of 223 ng $\sum$ $PBDE_6$/g lipid was found in the 2000–2003 period in the Western European and Others Group (WEOG), followed by 107 ng $\sum$ $PBDE_6$/g lipid in the Asia-Pacific Group in the 2016–2019 period. The WEOG group comprises Australia, Israel, New Zealand, and the USA as "Others." Therefore, with regard to the different history of use of PBDE, this UN Regional Group was further split into two subgroups "Western European countries" and "Other countries." Whereas 21 country results from the subgroup "Western European countries" had $\sum$ $PBDE_6$ concentrations in the range 0.76–7.72 ng/g, five country results from the subgroup "Other countries" were in the range 5.16–223 ng/g, with highest concentrations in the 2000–2003 period in both subgroups (for a more detailed discussion, see the end of this Sect. 3.1).

Based on the compilation of results in Table 2, a **general estimation of time trends** is quite difficult. In particular in the Asia-Pacific Group, the Group of Latin American and Caribbean Countries and in the Western European and Others Group, median or maximal concentrations don't appear to have a continuous downward time trend. The median of all country results shows a decrease of about 50% from the 2.62 ng $\sum$ $PBDE_6$/g lipid in 2000–2003 period to 1.38 ng $\sum$ $PBDE_6$/g lipid in the 2016–2019 period. These are seen as current background levels as defined in Sect. 2.5 (*found in the absence of specific sources and therefore not attributable to a known exposure*). As explained, time trends should be seen in context with the concentration range: If high levels are found, sources might be detected, which could be eliminated. Though, at "background levels", other factors, e.g., contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally. Therefore, conclusions on time trends in the different UN regional groups or globally cannot easily be drawn. Generally, a more precise approach for the assessment of temporal trends is based on consideration of results only from countries with repeated participation in the studies (see Sect. 3.2). For comparison of data in a literature review and conclusions on temporal tendencies, see Fürst (2023) and Fång et al. (2015).

Figure 1 illustrates the country results for the **African region**. As in the following figures for other regions, the 4-year periods between 2000 and 2019 are shown in different colors; results for Africa are available for the three periods between 2008 and 2019. All countries had at all times $\sum$ $PBDE_6$ concentrations approximately

Table 2 Minimum, median, and maximum concentrations of $\sum PBDE_6$ in human milk (expressed as ng $\sum PBDE_6$/g lipid) in the five UN regional groups for the five periods between 2000 and 2019 based on 124 country results (see Sect. 2.3) with the number of country results [*N*] in the respective period

	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019	All (2000–2019)
African Group						
N	0	0	11	2	15	28
Min			0.67	1.10	0.39	0.39
Median			1.60	1.15	1.38	1.41
Max			2.83	1.20	2.31	2.83
Asia-Pacific Group						
N	3	3	8	0	12	26
Min	2.70	1.41	0.28		0.41	0.28
Median	2.93	3.61	1.24		3.85	2.81
Max	7.18	4.97	26.2		107	107
Group of Latin American and Caribbean countries						
N	1	1	9	2	9	22
Min			0.65	4.29	0.39	0.39
Median	0.74	21.9	8.87	5.85	1.62	4.66
Max			62.7	7.40	13.8	62.7
Eastern European Group						
N	8	3	2	7	2	22
Min	0.66	0.83	0.52	0.59	0.49	0.49
Median	0.76	0.87	0.65	0.76	0.55	0.76
Max	1.87	1.23	0.78	1.78	0.60	1.87
Western European and Others Group (WEOG)						
N	12	4	1	4	5	26
Min	1.97	1.89		1.05	0.76	0.76
Median	3.16	3.23	5.16	3.56	1.04	2.75
Max	223	4.24		6.83	1.73	223
WEOG-subgroup "Western European countries"						
N	10	4	0	2	5	21
Min	1.97	1.89		1.05	0.76	0.76
Median	2.92	3.23		1.30	1.04	2.56
Max	7.72	4.24		1.55	1.73	7.72
WEOG-subgroup "other countries"						
N	2	0	1	2	0	5
Min	11.8			5.57		5.16
Median	117		5.16	6.20		6.83
Max	223			6.83		223
All						
N	24	11	31	15	43	124
Min	0.66	0.83	0.28	0.59	0.39	0.28
Median	2.62	2.56	2.01	1.10	1.38	1.61

(continued)

Table 2 (continued)

	2000–2003	2004–2007	2008–2011	2012–2015	2016–2019	All (2000–2019)
80% Percentile	4.22	4.24	5.16	4.55	4.78	4.73
Max	223	21.9	62.7	7.40	107	223

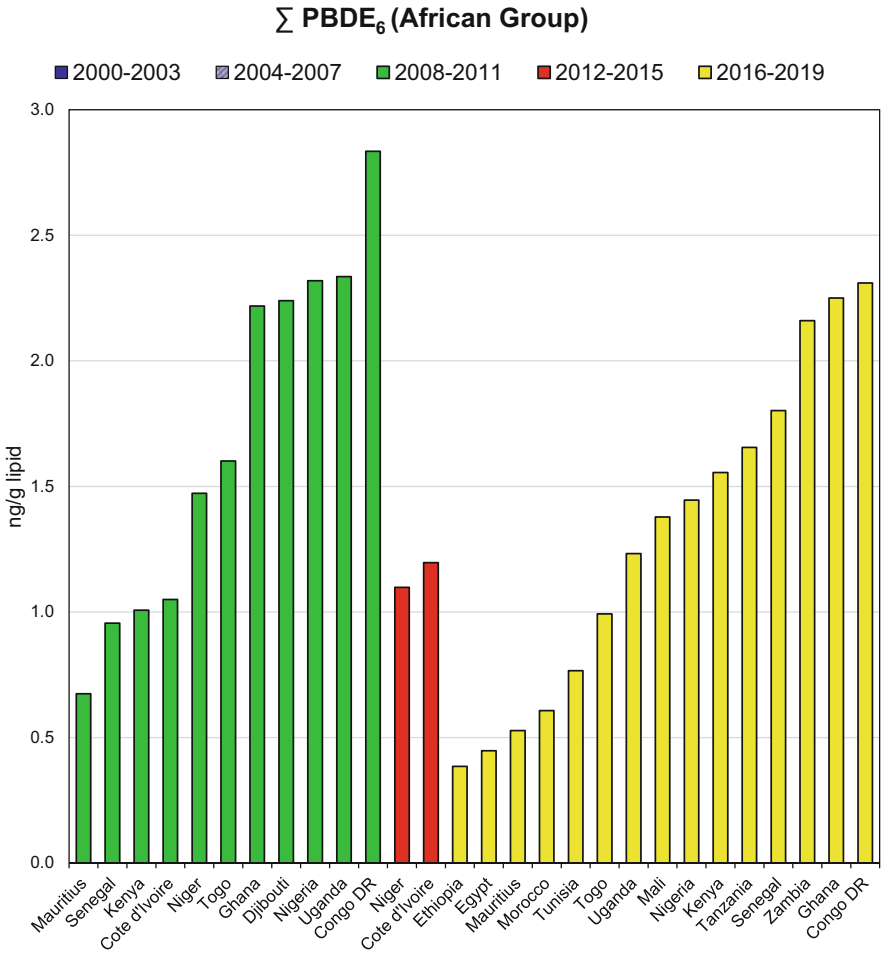

Fig. 1 Concentrations of ∑ $PBDE_6$ in human milk from countries in the African Group with submission of samples in three periods between 2008 and 2019 (country results expressed as ng ∑ $PBDE_6$/g lipid)

between 0.5 and 3 ng/g lipid, with quite comparable ranges between the 2008–2011 period (median of 11 samples: 1.60 ng/g lipid, range 0.67–2.83 ng/g) and the 2016–2019 period (median of 15 samples: 1.38 ng/g lipid, range 0.39–2.31 ng/g). For the assessment of time trends in ten countries with repeated participation (Democratic Republic of Congo, Côte d'Ivoire, Ghana, Kenya, Mauritius, Niger, Nigeria, Senegal, Togo, Uganda), see Sect. 3.2.1.

In the **Asia-Pacific Group**, most samples had concentrations below 5 ng ∑ $PBDE_6$/g lipid (Fig. 2), in many cases around 0.5–1 ng/g lipid. Five samples from the 2016–2019 period and two samples from earlier periods had significantly higher

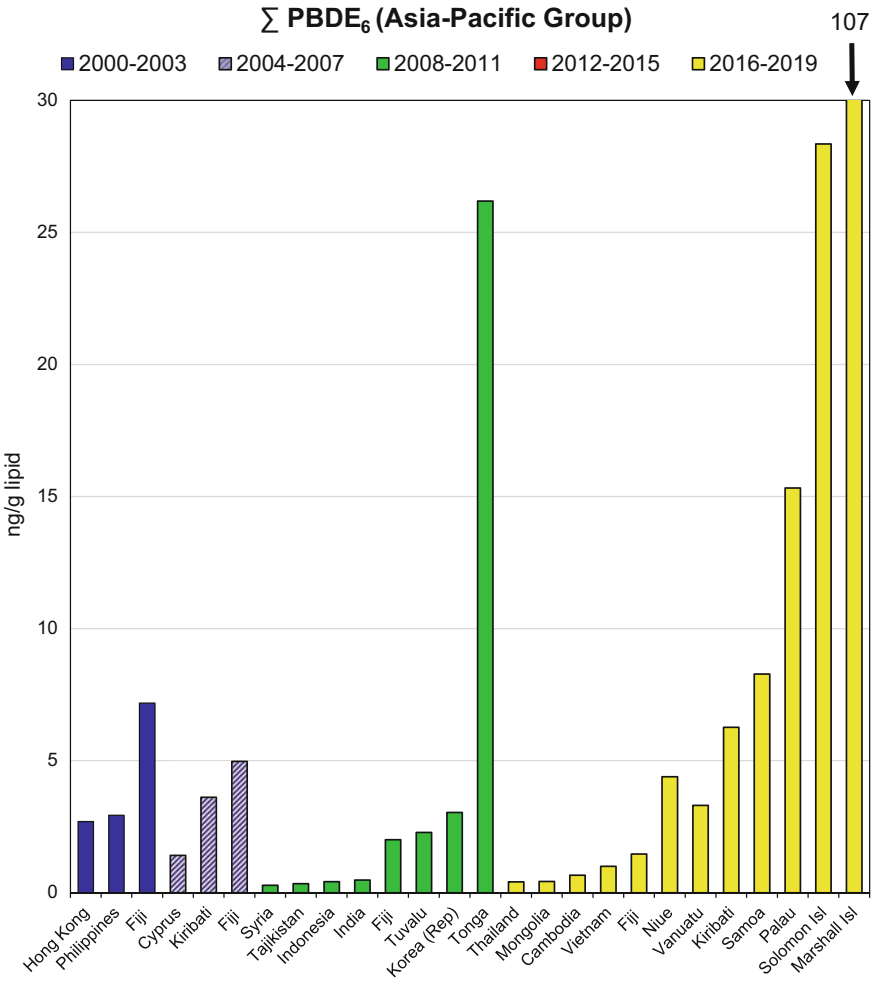

Fig. 2 Concentrations of ∑ $PBDE_6$ in human milk from countries in the Asia-Pacific Group with submission of samples in four periods between 2000 and 2019 (country results expressed as ng ∑ $PBDE_6$/g lipid)

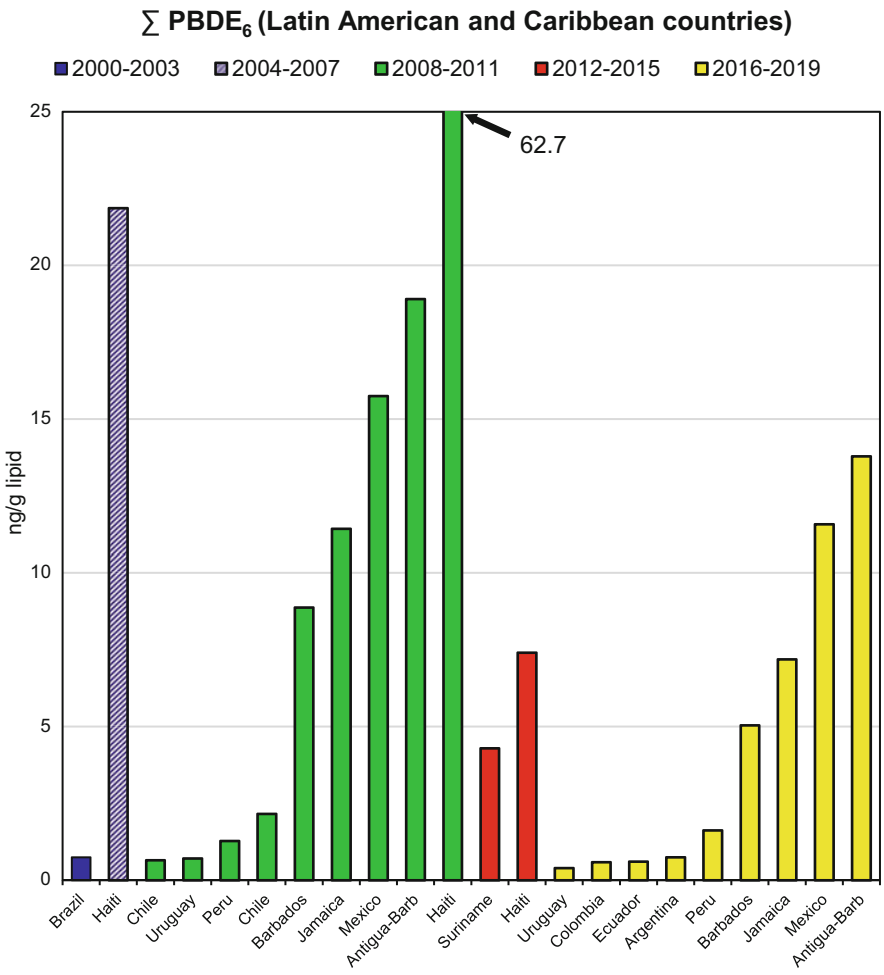

Fig. 3 Concentrations of ∑ $PBDE_6$ in human milk from countries in the Group of Latin American and Caribbean Countries with submission of samples in five periods between 2000 and 2019 (country results expressed as ng ∑ $PBDE_6$/g lipid)

levels, >5 ng/g lipid, with a maximum of 107 ng ∑ $PBDE_6$/g found in 2019 in the Marshall Islands. For only two countries, data for the assessment of time trends from repeated participation are available (Fiji; Kiribati), see Sect. 3.2.2.

Figure 3 illustrates the ∑ $PBDE_6$ results with the period of participation between 2000 and 2019 indicated for **Latin American and Caribbean countries**. A range between 0.39 ng ∑ $PBDE_6$/g lipid (Uruguay, 2019) and 62.7 ng ∑ $PBDE_6$/g lipid (Haiti, 2011) was found. For the assessment of time trends in eight countries with repeated participation (Antigua and Barbuda, Barbados, Chile, Haiti, Jamaica, Mexico, Peru, and Uruguay), see Sect. 3.2.3.

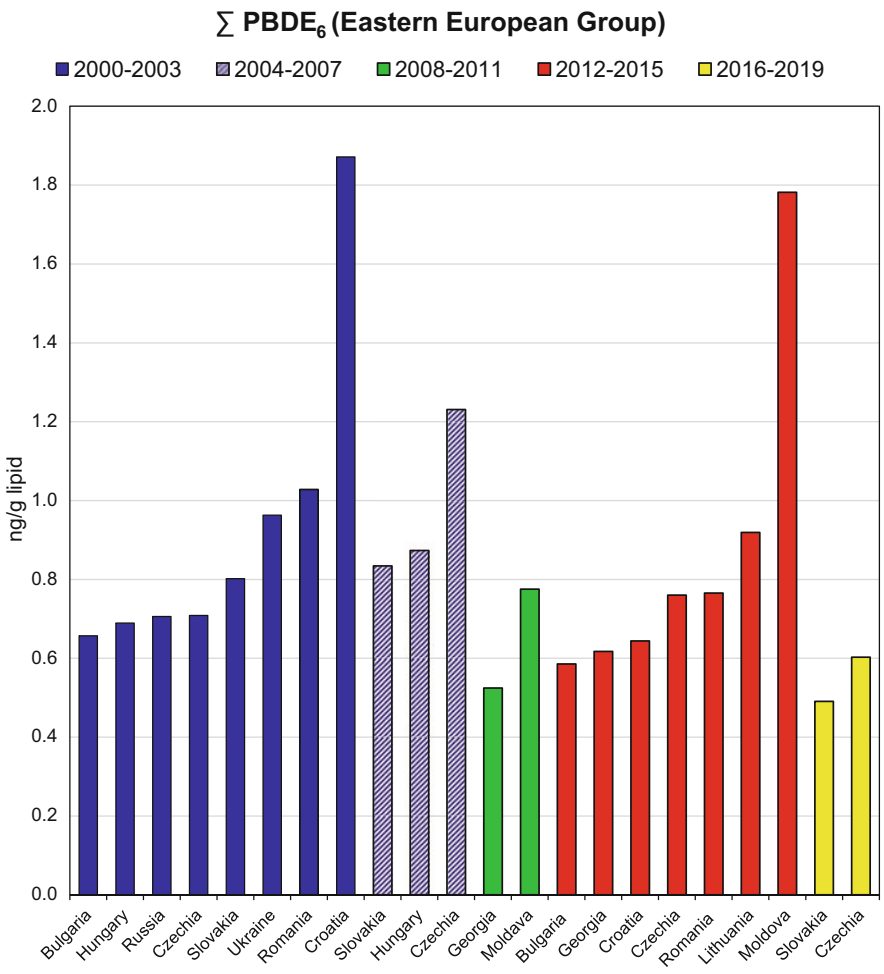

Fig. 4 Concentrations of ∑ $PBDE_6$ in human milk from countries in the Eastern European Group with submission of samples in five periods from 2000 to 2019 (country results expressed as ng ∑ $PBDE_6$/g lipid)

All **Eastern European countries** had at all times ∑ $PBDE_6$ concentrations approximately between 0.5 and 2 ng/g lipid (Fig. 4). For the assessment of time trends in eight countries with repeated participation (Bulgaria, Croatia, Czech Republic, Georgia, Hungary, Moldova, Romania, and Slovakia), see Sect. 3.2.4.

As explained above, the **Western European and Others Group** (WEOG) includes Australia, Israel, New Zealand, and the USA (being informally a member) as "Others" (see section "regional distribution" in Malisch et al. 2023a). In the period 2000–2003, ∑ $PBDE_6$ concentrations in human milk from Western European countries were in the range between 2.0 ng/g lipid (Belgium, 2002) and 7.7 ng/g lipid (Ireland, 2001), with downward trends until the 2016–2019 period (range

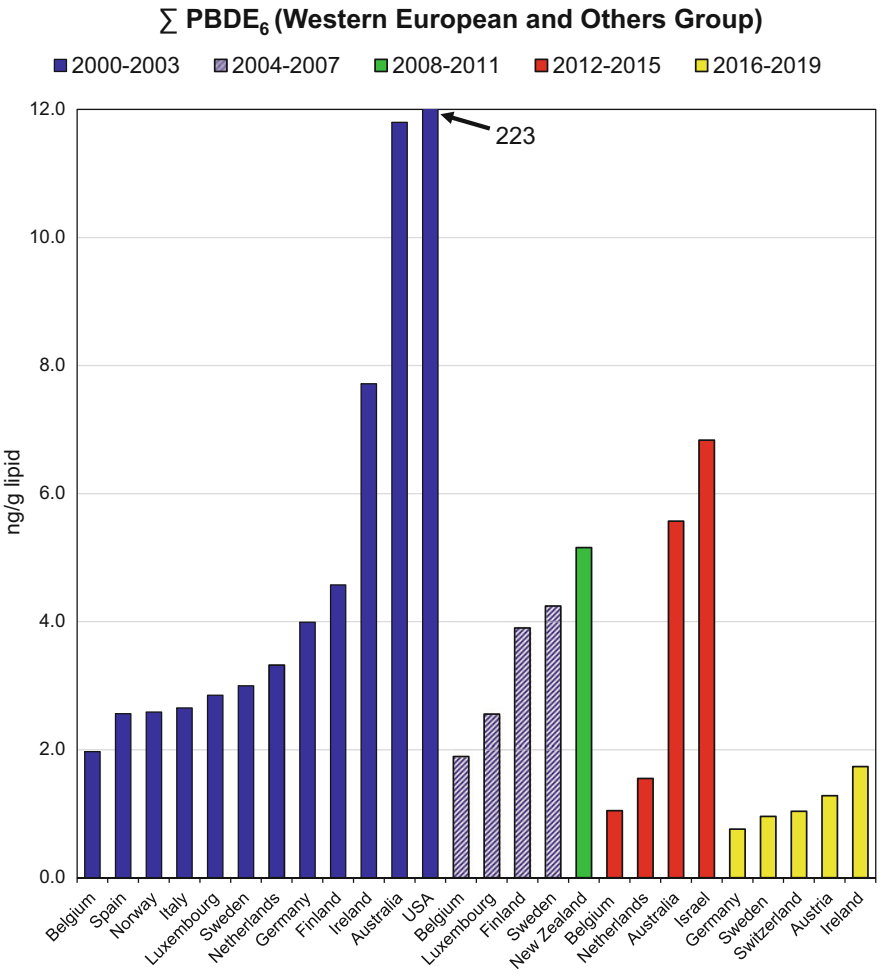

Fig. 5 Concentrations of ∑ $PBDE_6$ in human milk from countries in the Western European and Others Group with submission of samples in five periods from 2000 to 2019 (country results expressed as ng ∑ $PBDE_6$/g lipid)

between 0.76 ng/g lipid [Germany, 2019] and 1.73 ng/g lipid [Ireland, 2019]). In New Zealand, Australia, and Israel the ∑ $PBDE_6$ concentrations were in the range between 5.16 ng/g lipid (New Zealand, 2011) and 11.8 ng/g lipid (Australia, 2002). As highest ∑ $PBDE_6$ concentration observed in all countries between 2000 and 2019, 223 ng ∑ $PBDE_6$/g lipid was the country result for samples collected in 2003 in the USA (as median of two pooled samples from North Carolina [92.6 ng/g lipid] and in California [352 ng/g lipid]) (Fig. 5).

These findings of the high PBDE levels in human milk from the USA in 2003 are in line with reports that levels of PBDE in US residents were 3–25 times higher than

those of individuals in Europe at that time. The levels of PBDE in human tissue samples from California women were the highest reported then. The high PBDE levels (up to 462 ng/g lipid for the sum of 5 PBDE [*BDE-47, BDE-99, BDE-100, BDE153, and BDE-154*] in human breast adipose tissues) could be partially explained by the fact that California regulations required all furnishings to pass flammability tests for fire safety (She et al. 2002). The presence of few samples with unusually high PBDE levels in human milk from the Pacific Northwest of the US and Canada suggested, as did a number of other studies of human blood (e.g., Petreas et al. 2003), that the frequency of high (>300 ng/g lipid) levels for the sum of 12 PBDE was greater than predicted by a lognormal distribution (She et al. 2007). These 12 PBDE comprised the five most important congeners of the 2002 study, which made up about 90–95% of the sum of 12 PBDE. The other seven congeners were BDE-28, BDE-32, BDE-66, BDE-71, BDE-85, BDE-183, and BDE-209. The sum of the six recommended analytes (BDE 47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE 175/183 [co-eluting]) and the sum of these six PBDE plus BDE-209 (UNEP 2019) was nearly the same as the sum of the five congeners (median contribution to the sum of 12 PBDE: $\sum$ $PBDE_5$ 92.4%; $\sum$ $PBDE_6$ 93.0%; $\sum$ $PBDE_{6+BDE\text{-}209}$ 94.0%). Thus, the recommended analytes cover most of $\sum$ $PBDE_{12}$; the remaining ones represent very low levels.

The determination of up to 13 PBDE in 47 individual human milk samples from nursing mothers in the USA found a variation of the concentrations of the sum of PBDE congeners from 6.2 to 419 ng/g lipid. These findings showed extremely elevated levels (10–100 times) in many participants compared with contemporaneous studies reported in Europe. Comparing the legal situation at that time, it was noted that use of PBDE was permitted in the USA but had already been restricted in some European countries (Schecter et al. 2003).

California flammability standards became common for residential, business, and institutional upholstered furniture across North America. The flame retardant primarily used in polyurethane furniture foam through the mid-2000s was Penta-BDE. The use of Penta-BDE was banned in the European Union in 2004 and discontinued in the USA in 2005. In 2009, tetra-BDE and penta-BDE were added to the Stockholm Convention on POPs. By the time of these restrictions, millions of kilograms of Penta-BDE had been applied to upholstered furniture in order to meet flammability standards (Charbonnet et al. 2020). For a review of the exposure of Americans to PBDE at that time, see Lorber (2008), for discussion of toxicological concerns, see Birnbaum and Staskal (2004), and for decreasing PBDE levels in breast milk in California after implementation of new governmental regulatory policies on PBDE, see Guo et al. (2016). In view of the high amounts of PBDE produced commercially as flame retardants in combination with their environmental persistence, it can be expected that much of these PBDE may still be released into the environment in the near future as a result of disposal when the products containing them reach their end of life.

3.2 Temporal Tendencies for the Sum of Six PBDE Congeners Derived from Countries with Repeated Participation

One of the objectives of the WHO/UNEP-coordinated human milk exposure studies was to generate comparable and consistent monitoring data on the presence of these POPs in order to identify trends in levels. To provide reliable monitoring information for the Parties to the Stockholm Convention, as a quantitative objective for temporal studies *The Guidance Document on the Global Monitoring Plan* (GMP) proposed the ability to detect a 50% decrease in the levels of POPs within a 10-year period. However, there is no stipulation of a quantitative goal for the rate of decline in POPs levels. Clearly, the Convention's objectives are either to eliminate or to reduce production, use and releases, depending on the annex where a chemical is listed, but the rate of the decline is nowhere specified or required (UNEP 2015, 2019).

For assessment of this goal, the exponential trends calculated by the Theil-Sen method comprise the overall decrease rates per 10 years. *Trends* can be derived if the trend test (significance of the Theil-Sen estimator) is positive on 95% confidence level of significance (i.e., p-values < 0.05). As simulations show that Theil-Sen p is never below 0.05 for fewer than 5 data points and as for most countries fewer than 5 data points were available, statistically significant trends could be derived only for few countries and for most UN regions (combining data from countries). If the minimum of five data points is not available allowing to derive statistically significant temporal trends, the existing data can indicate decreasing or increasing *tendencies* in POP concentrations.

To minimize possible sources of variation for time trend analysis of POPs, the concept of the WHO/UNEP-coordinated exposure studies has two basic elements: (1) preparation of pooled samples from a number of individual samples considered to be representative for a country or region/subgroup; (2) analysis by a reference laboratory.

The above Sect. 3.1 shows a considerable variation of the PBDE concentrations in human milk from 80 countries in the five periods between 2000 and 2019, depending on the inclusion of the individual countries in a certain round. As a result, a general estimation of time trends among the different UN regional groups and globally is difficult from a statistical point of view. Therefore, the following temporal trends are assessed based on results only from countries with a repeated participation in the different surveys. For 36 of the 80 countries, PBDE data for 90 pooled samples from participation in two or more rounds are available for this purpose. As explained above, time trends should be seen in context with the concentration range: If high levels are found, sources might be detected, which could be eliminated. In the range of background contamination, other factors cannot be influenced locally, and the concentrations might fluctuate over time.

3.2.1 African Group

As shown above in Sect. 3.1, all African countries always had $\sum$ $PBDE_6$ concentrations in the range of background contamination, approximately between 0.5 and 3 ng/g lipid, with quite comparable ranges between the 2008–2011 period

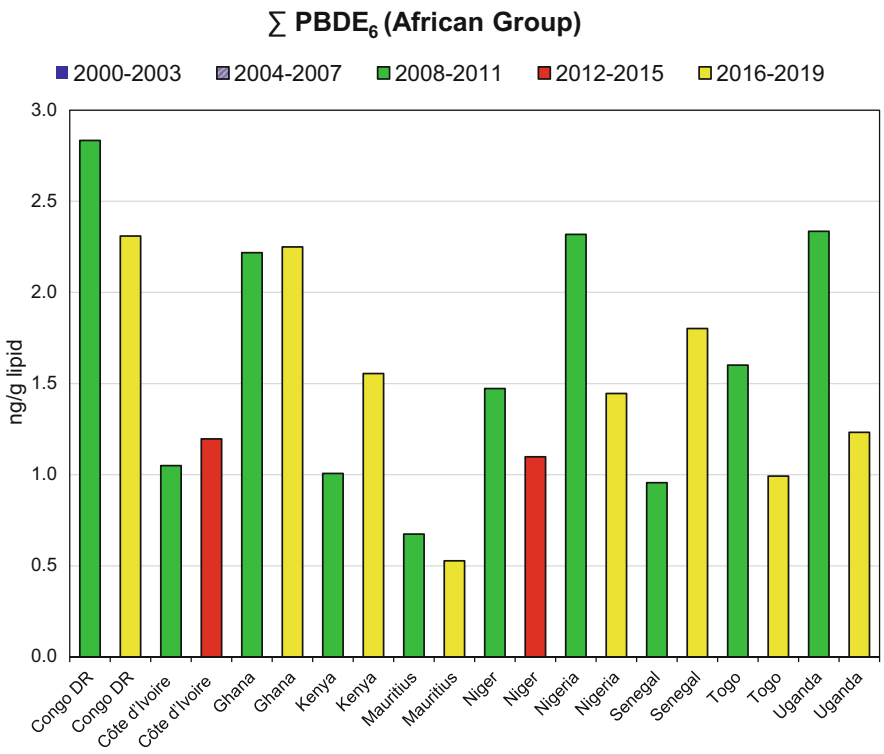

Fig. 6 Overview of the changes in ∑ $PBDE_6$ concentrations (ng ∑ $PBDE_6$/g lipid) over time in African countries with repeated participation between 2000 and 2019

and the 2016–2019. Figure 6 (for aggregated data) shows the variation over time at these background levels for ten countries with data for two sampling periods. Figure 7 (comprising all individual pooled samples) shows the resulting Theil-Sen exponential time trends for the African Group. The overall decrease rates (%) per 10 years are compiled in Table 3. All temporal tendencies were statistically not significant, as well for the countries as the regional trend derived by pooling of the individual samples from all countries.

In Fig. 7, results are shown on annual basis for the period 2008–2019, Fig. 6 uses 4-year periods, and Table 3 presents data for decrease (or increase as negative results) over 10 years. The annual basis in Fig. 7 combines different numbers of samples for the respective years, e.g. one sample in 2008 (2.32 ng/g), six samples in 2009 (range 0.96–2.83 ng/g), two samples in 2015 (1.10 ng/g; 1.20 ng/g) or three samples in 2019 (range 1.45–2.25 ng/g). The time interval between first and last participation illustrated in Fig. 6 covers a range between 5 years (Côte d'Ivoire, 2010 and 2015) and 10 years (Nigeria, 2008 and 2018), which is normalized into tendencies over 10 years as presented in Table 3. Therefore, a direct comparison of results of Table 3 with the illustrations in Figs. 6 and 7 is difficult.

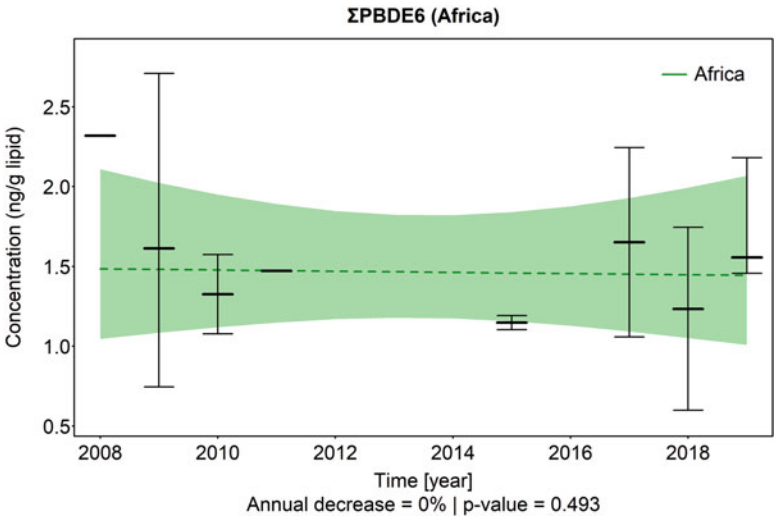

Fig. 7 Theil-Sen exponential trends of ∑ PBDE6 concentrations in human milk of countries in the African Group (ng ∑ $PBDE_6$/g lipid). The thick lines in the middle show the median value, the whiskers show 5th and 95th percentiles

Table 3 Overall decrease rates per 10 years (%) of ∑ $PBDE_6$ concentrations in human milk of countries in the African Group (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease rate (%) per 10 years	Trend *p*-value overall
Congo (DR)[a]	22.6	1.000
Côte d'Ivoire[a]	–30.0	1.000
Ghana[a]	–1.5	1.000
Kenya[a]	–54.5	1.000
Mauritius[a]	23.8	1.000
Niger[a]	51.9	1.000
Nigeria[a]	34.9	1.000
Senegal[a]	–102.3	1.000
Togo[a]	49.5	1.000
Uganda[a]	50.8	1.000
African Group	2.5	0.493

[a]In all periods <3 ng ∑ $PBDE_6$/g lipid

As a conclusion for the African Group, ∑ $PBDE_6$ concentrations fluctuate over time at background levels, but remained constantly in this low range. Overall rates over 10 years can be calculated for individual countries but are of little relevance in cases where background levels might be leveling out. This conclusion is more clearly visualized in Fig. 7.

3.2.2 Asia-Pacific Group

Some countries of the Asia-Pacific group did not participate repeatedly. In addition, some countries of the Pacific region did not send sufficient sample amounts necessary for the application of the various analytical methods to be applied: There is no multi-method for simultaneous determination of *all* POPs of interest for the Stockholm Convention that could limit the total amount of human milk needed for this combined POPs analysis. Consequently, PBDE data for assessment of time trends are available only for two countries (Fiji and Kiribati). Figure 8 shows the results for these two countries; Fig. 9 visualizes the summarizing time trend; and Table 4 lists the decrease rates over 10 years.

For Fiji, results from four periods (with two samples submitted in 2006) are available showing a continuous downwards trend from 2002 (7.2 ng ∑ $PBDE_6$/g) to 2019 (1.5 ng ∑ $PBDE_6$/g). These five available data allow to determine a statistically significant decrease rate for Fiji, whereas the two samples sent by Kiribati in 2006, respectively, 2019 do not allow to derive statistically significant decrease rates. Based on these seven results from these two countries, statistically significant group rates can be calculated—however, certainly, more results from more countries

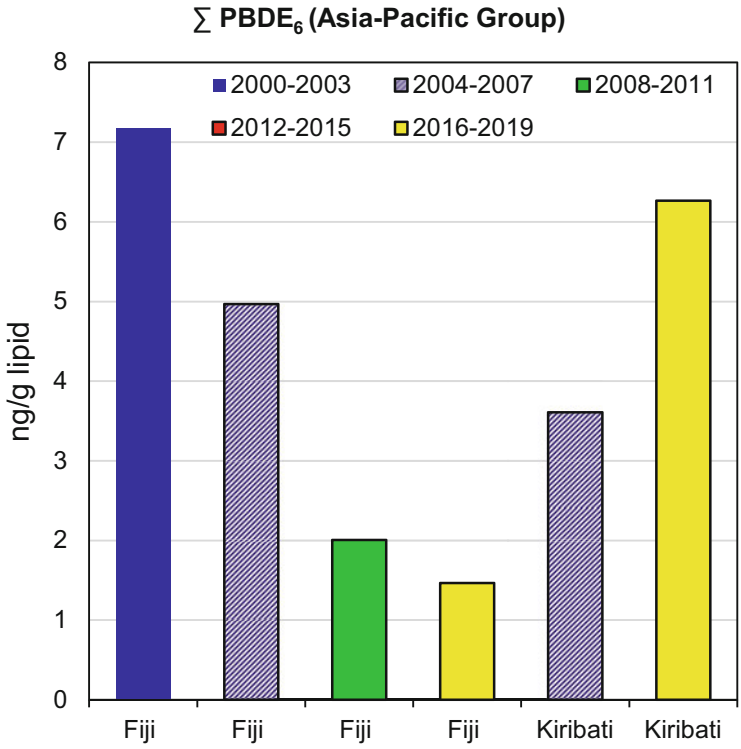

Fig. 8 Overview of the changes in ∑ $PBDE_6$ concentrations (ng ∑ $PBDE_6$/g lipid) over time in countries of the Asia-Pacific Group with repeated participation between 2000 and 2019

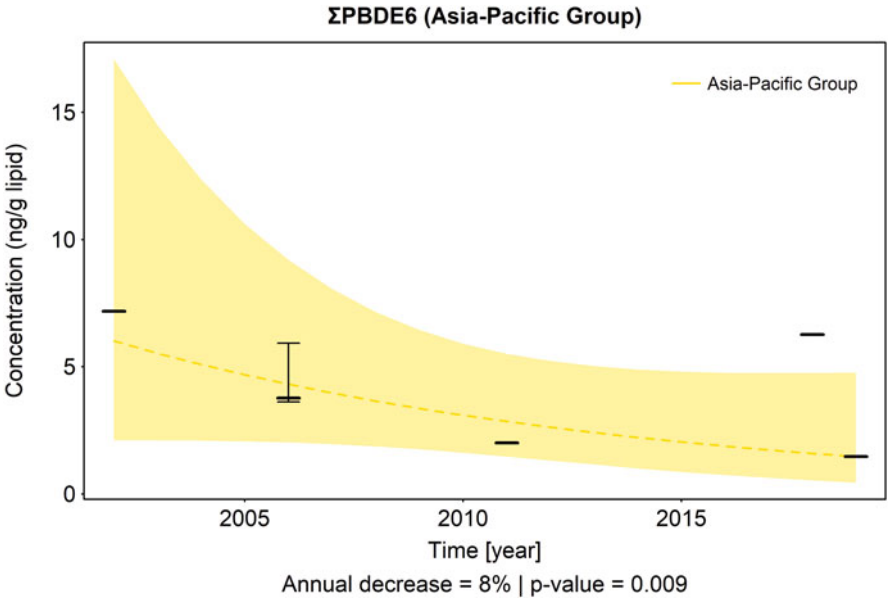

Fig. 9 Theil-Sen exponential trends of ∑ PBDE6 concentrations in human milk of countries in the Asia-Pacific Group (ng ∑ $PBDE_6$/g lipid). The thick lines in the middle show the median value, the whiskers show 5th and 95th percentiles

Table 4 Overall decrease rates (%) per 10 years of ∑ $PBDE_6$ concentrations in human milk of countries in the Asia-Pacific Region (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease rate (%) per 10 years	Trend *p*-value overall
Fiji	66.9	0.004
Kiribati	–58.4	1.000
Asia-Pacific Group[a]	*56.4*	*0.009*

[a]More country results are necessary before conclusions on this group can be drawn

would be necessary before conclusions for the whole Asia-Pacific Group can be drawn.

3.2.3 Group of Latin American and Caribbean Countries (GRULAC)

Figure 10 illustrates the time trends of ∑ $PBDE_6$ concentrations in human milk of eight Latin American and Caribbean countries, Fig. 11 for this UN region.

In Haiti, the ∑ $PBDE_6$ concentrations before 2011 (in 2004 with 21.9 ng/g lipid) and after 2011 (in 2015 with 7.4 ng/g lipid) were considerably lower than the maximum found in the 2011 sample (62.7 ng/g lipid). The increasing trend from 2004 to 2011 and decreasing trend to 2019 might be discussed with the voluntarily withdrawal of Penta-BDE and Octa-BDE formulations from the US marketplace by their manufacturers at the end of 2004 as a result of findings of high ∑ $PBDE_6$

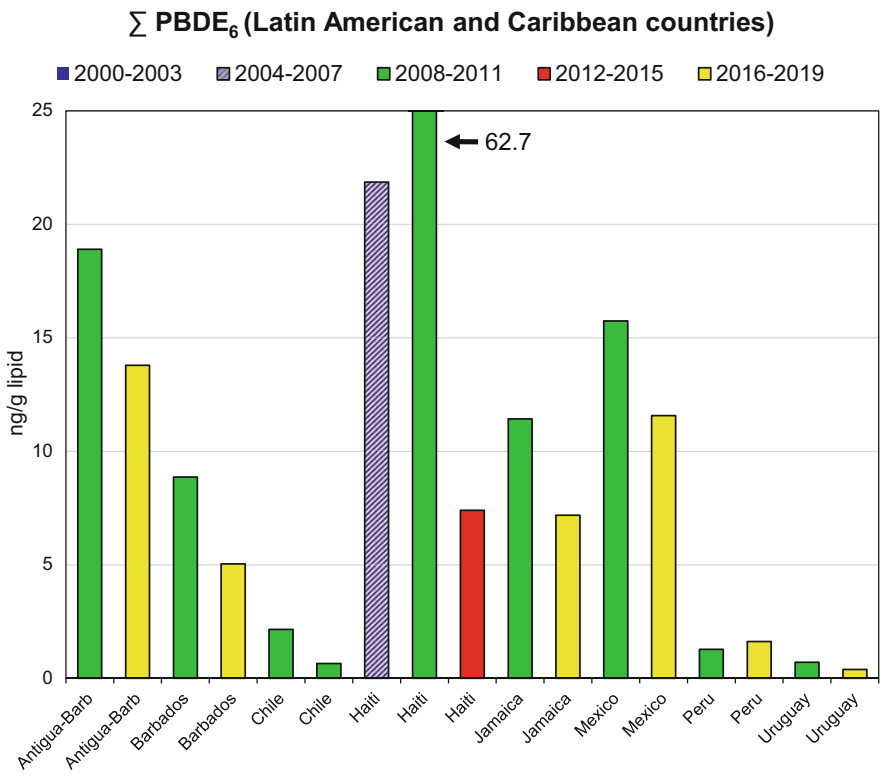

Fig. 10 Overview of the changes in ∑ $PBDE_6$ concentrations (ng ∑ $PBDE_6$/g lipid) over time in Latin American and Caribbean countries with repeated participation between 2000 and 2019

concentrations in human milk from the USA (see results and discussion of "Western European and Others Group" in Sect. 3.1). Therefore, the up and down in Haiti might be explainable with the delay, before the withdrawal of penta- and octa-BDE from the US marketplace became effective.

Downtrends from the 2008–2011 period to the 2016–2019 period were observed in Antigua and Barbuda, Barbados, Chile, Jamaica, and Mexico. In Peru, ∑ $PBDE_6$ remained quite constant around 1.5 ng/g lipid and in Uruguay around 0.5 ng/g lipid.

Table 5 compiles the decrease rates for 10 years. The limited number of samples did not allow to determine statistically significant time trends for countries. Furthermore, for the three countries below 3 ng ∑ $PBDE_6$/g lipid in all periods (Chile, Peru and Uruguay), the calculated decrease rates have a wide variation due to the fluctuation near background levels, as concluded already for African countries (Sect. 3.2.1). For the Group of Latin American and Caribbean Countries, the decrease rate over 10 years of nearly 50% is statistically significant.

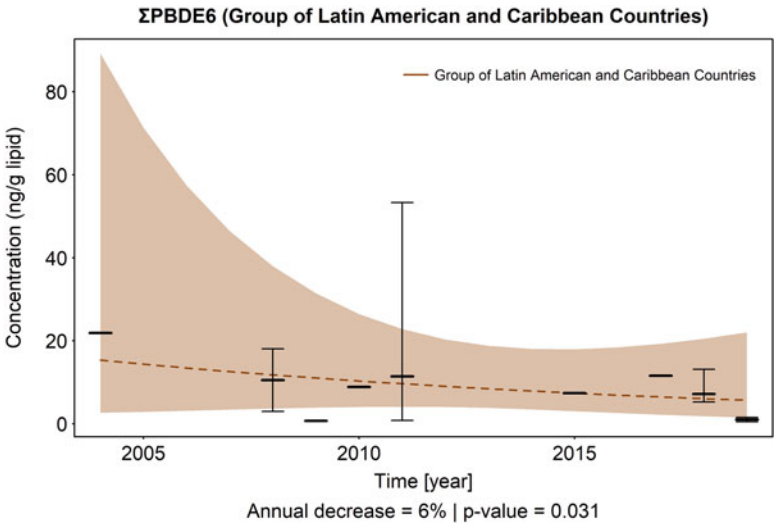

Fig. 11 Theil-Sen exponential trends of ∑ $PBDE_6$ concentrations in human milk of countries in the Group of Latin American and Caribbean Countries (ng ∑ $PBDE_6$/g lipid). The thick lines in the middle show the median value, the whiskers show 5th and 95th percentiles

Table 5 Overall decrease rates (%) of ∑ $PBDE_6$ concentrations per 10 years in countries of the Group of Latin American and Caribbean Countries (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease rate (%) per 10 years	Trend *p*-value overall
Antigua-Barbuda	27.1	1.000
Barbados	50.7	1.000
Chile[a]	98.2	1.000
Haiti	62.6	0.750
Jamaica	48.5	1.000
Mexico	40.1	1.000
Peru[a]	–35.6	1.000
Uruguay[a]	44.6	1.000
Group of Latin American and Caribbean Countries	48.5	0.031

[a]In all periods <3 ng ∑ $PBDE_6$/g lipid

3.2.4 Eastern European Group

As shown above in Sect. 3.1, all Eastern European countries had at all times ∑ $PBDE_6$ concentrations in the range of background contamination approximately between 0.5 and 2 ng/g lipid. Figure 12 illustrates the time trends of ∑ $PBDE_6$ concentrations in human milk of eight Eastern European countries with repeated participation (Bulgaria, Croatia, Czech Republic, Georgia, Hungary, Moldova, Romania, and Slovakia); Fig. 13 for this UN region; and Table 6 compiles the

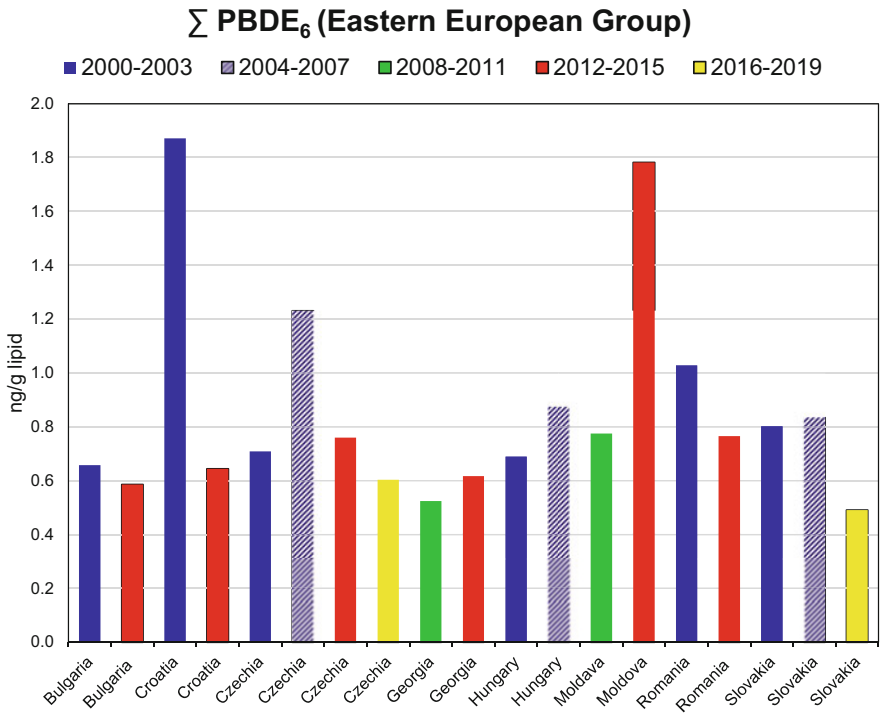

Fig. 12 Overview of the changes in ∑ $PBDE_6$ concentrations (ng ∑ $PBDE_6$/g lipid) over time in countries of the Eastern European Group with repeated participation between 2000 and 2019

decrease rates for 10 years. Due to limited data, the country-specific results are statistically not significant, however, pooling of the samples allows a trend for this group to be determined.

As a conclusion, the ∑ $PBDE_6$ concentrations tend to fluctuate at near background levels, but remain constant in this low range. For the Eastern European Group, the statistically significant decrease rate over 10 years was 15%.

3.2.5 Western European and Others Group (WEOG)

Figure 14 illustrates the time trends of ∑ $PBDE_6$ concentrations in human milk of eight countries of the Western European and Others Group (WEOG) and Fig. 15 for this UN regional group. Table 7 compiles the decrease rates for 10 years.

As shown above in Sect. 3.1, the two highest ∑ $PBDE_6$ concentrations were found in the 2000–2003 period in the USA (223 ng/g lipid as median of two samples) and then in Australia (11.8 ng/g lipid). Unfortunately, time trends for the highest PBDE concentration could not be derived from WHO/UNEP-coordinated exposure studies, as the USA has not participated after 2003 again. However, significant declines in PBDE levels between 2003–2005 and 2009–2012 were found in breast milk of California women: The concentration of the sum of PBDE

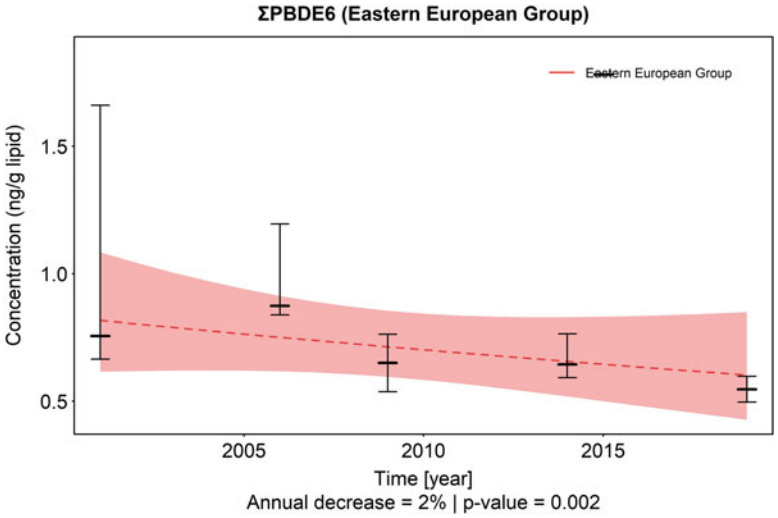

Fig. 13 Theil-Sen exponential trends of ∑ $PBDE_6$ concentrations in human milk of countries in the Eastern European Group (ng ∑ $PBDE_6$/g lipid). The thick lines in the middle show the median value, the whiskers show 5th and 95th percentiles

Table 6 Overall decrease rates (%) of ∑ $PBDE_6$ concentrations per 10 years of countries in the Eastern European Group (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease rate (%) per 10 years	Trend *p*-value overall
Bulgaria[a]	8.5	1.000
Croatia[a]	56.0	1.000
Czechia[a]	24.1	0.560
Georgia[a]	–38.6	1.000
Hungary[a]	–60.6	1.000
Moldova[a]	–300.1	1.000
Romania[a]	20.3	1.000
Slovakia[a]	23.9	0.500
Eastern European Group	15.5	0.002

[a]In all periods < 3 ng ∑ $PBDE_6$/g lipid

(Σ10PBDE = sum of BDE-28, -47, -66, -85, -99, -100, -153, -154, -183, and -209) over the ~7 year course declined by 39% (GeoMean = 67.8 ng/g lipid in 2003–2005; 41.5 ng/g lipid in 2009–2012) (Guo et al. 2016). In Australia, ∑ $PBDE_6$ concentrations in human milk decreased by 53% between 2002 and 2013.

Western European countries were in the range between 2.0 and 7.7 ng/g lipid. For four Western European countries, data are available for the 2000–2003 period as well as the 2004–2007 period (Belgium, Finland, Luxembourg, and Sweden). Over this time, the ∑ $PBDE_6$ concentrations remained quite constant. In comparison with these two periods, downward trends were observed afterward to the 2012–2015 and

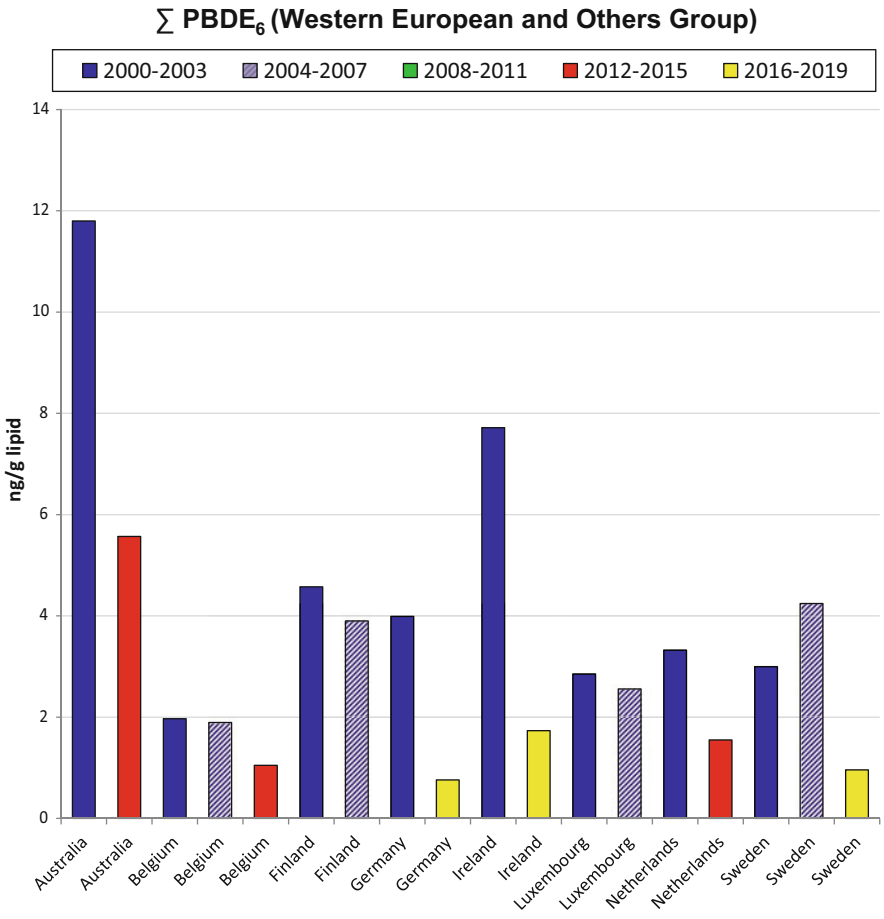

Fig. 14 Overview of the changes of ∑ $PBDE_6$ concentrations (ng ∑ $PBDE_6$/g lipid) over time in countries of the Western European and Others Group with repeated participation between 2000 and 2019

2016–2019 surveys in all countries participating in these periods (Fig. 14). These observations are in line with the legal situation in the EU: A directive of the European Parliament and the Council of 2003 restricted the concentration of penta-BDE and octa-BDE in articles to 0.1% and entered into force in 2004 (Directive 2003/11/EC).

Due to lack of sufficient data, for most countries the decrease rates over 10 years for countries are statistically not significant (Table 7). Statistically significant is the decrease rate over 10 years of 60% in Germany and of 48% for this group of countries.

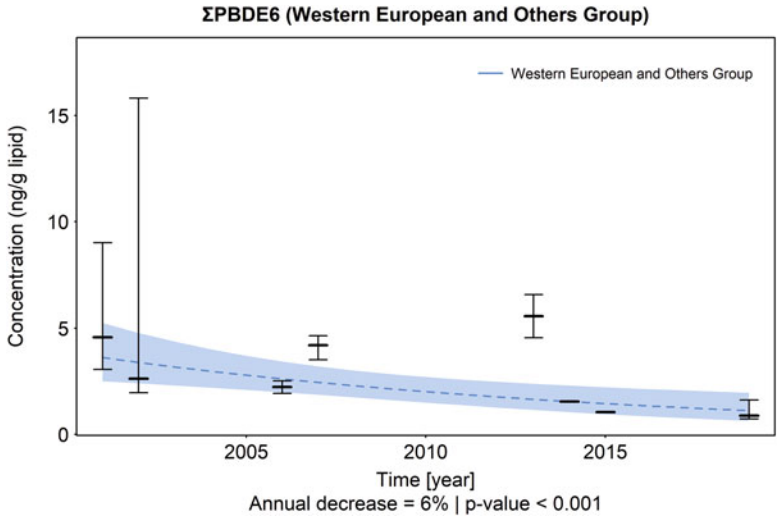

Fig. 15 Time trends of ∑ $PBDE_6$ concentrations in human milk of countries of the Western European and Others Group using the Theil-Sen method (ng ∑ $PBDE_6$/g lipid). The thick lines in the middle show the median value, the whiskers show 5th and 95th percentiles

Table 7 Overall decrease rates (%) of ∑ $PBDE_6$ concentrations per 10 years in countries of the Western European and Others Group (calculated by the Theil-Sen method)

Country	Overall decrease rate (%) per 10 years	Trend *p*-value overall
Australia	50.4	0.500
Belgium[a]	38.0	0.063
Finland[b]	14.7	0.500
Germany	59.8	0.008
Ireland	55.4	0.500
Luxembourg[a,b]	23.9	1.000
Netherlands	44.4	1.000
Sweden	47.0	0.500
Western European and Others Group	47.9	<0.001

[a]In all periods <3 ng ∑ $PBDE_6$/g lipid
[b]Data points from the 2000–2003 and 2004–2007 period

3.2.6 Global Level

The exponential trends of ∑ $PBDE_6$ concentrations in human milk derived by the Theil-Sen method worldwide are illustrated in Fig. 16. At the global level calculated from 36 countries, the statistically significant overall decrease rate per 10 years was 32% calculated by the Theil-Sen method, respectively, 48% by the median method (Table 8).

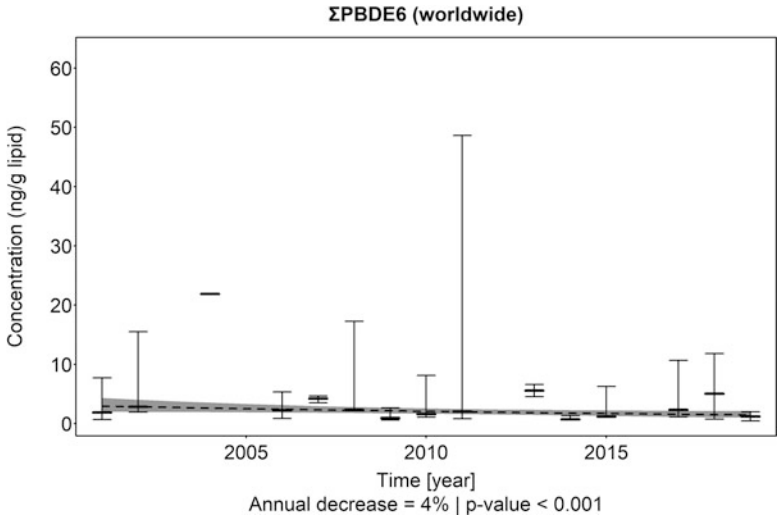

Fig. 16 Global time trends of ∑ $PBDE_6$ concentrations in human milk using the Theil-Sen method (ng ∑ $PBDE_6$/g lipid). The thick lines in the middle show the median value, the whiskers show 5th and 95th percentiles

Table 8 Overall global decrease rates (%) of ∑ $PBDE_6$ concentrations in human milk (expressed as ng ∑ $PBDE_6$/g lipid)

	No of countries	Overall decrease rate (%) per 10 years		Trend *p*-value overall
		Theil-Sen method	Median method	
Global	36	32.3	48.0	<0.001

3.3 Decabromodiphenyl Ether (BDE-209)

As explained in the introduction chapter, the number of target analytes in the global human milk studies gradually expanded as new POPs were listed in the annexes of the Stockholm Convention. BDE-209 was listed in 2017 and included in the analysis of human milk samples for the 2016–2019 period (Malisch et al. 2023a).

BDE-209 concentrations in 40 pooled human milk samples from 39 countries in this period were in the range between <0.06 and 5.92 ng/g, with a median of 0.21 ng/g and a 90%-percentile of 1.53 ng/g. The median of the contribution of BDE-209 to the sum of 7 PBDEs (∑ $PBDE_7$ = ∑ $PBDE_6$ + BDE-209) was 13% but ranged from 3 to 66% (75% quantile: 21%). This large difference in contribution of BDE-209 to ∑ $PBDE_7$ could possibly be explained by the difference in local production and use of different commercial PBDE mixtures as flame retardants.

In nearly all 14 countries from ***Africa***, the BDE-209 concentrations were below 0.4 ng/g lipid, in these cases with a contribution between 6 and 29% to ∑ $PBDE_7$. As maximum, 1.51 ng BDE-209/g lipid was found in Tunisia exceeding the ∑ $PBDE_6$

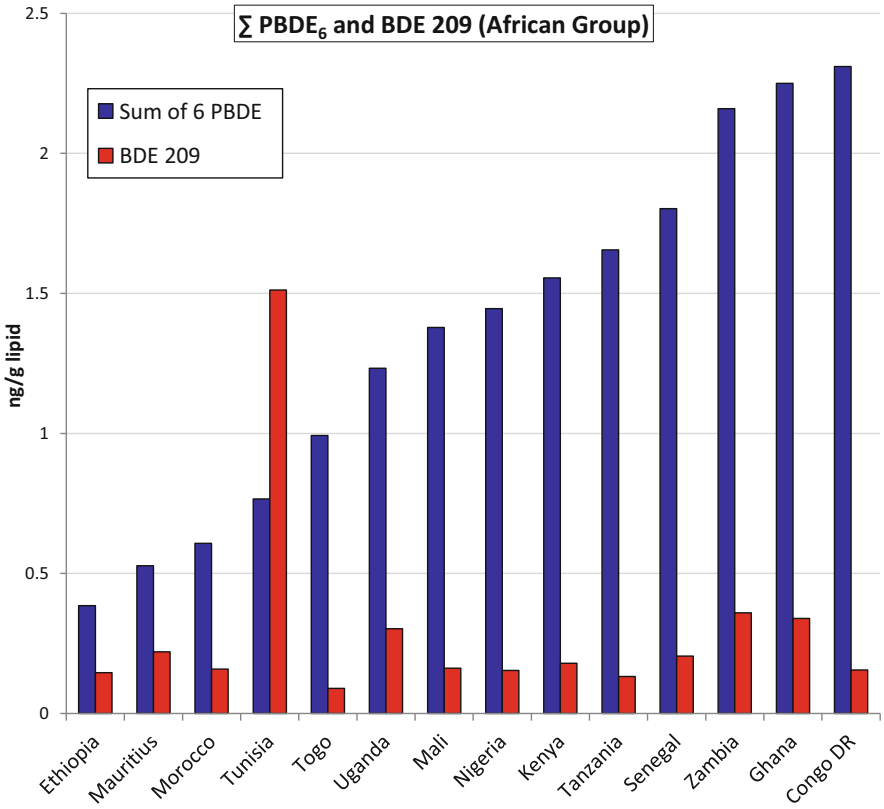

Fig. 17 Concentration of BDE-209 and ∑ $PBDE_6$ in human milk from countries in the African Group for the period 2016–2019 (expressed as ng ∑ $PBDE_6$/g lipid and ng BDE-209/g lipid, respectively)

concentration. In these 14 countries, ∑ $PBDE_6$ levels were in the range of background contamination between 0.39 and 2.31 ng/g lipid (Fig. 17).

In four **Asian** countries of the ***Asia-Pacific Group***, the BDE-209 concentrations were below 0.3 ng/g lipid; with ∑ $PBDE_6$ levels in the range of background contamination below 1 ng/g lipid. With increasing ∑ $PBDE_6$ levels in the **Pacific** region, also higher BDE-209 concentrations were found, with a maximum of 5.92 ng BDE-209/g lipid in Marshall Islands, contributing 5% to ∑ $PBDE_7$ (113 ng/g lipid). In Palau, BDE-209 results (3.31 ng/g lipid) contributed 18% to ∑ $PBDE_7$ (15.3 ng/g lipid) (Fig. 18).

In most ***Latin American and Caribbean countries***, BDE-209 concentrations were below 1 ng/g lipid; with a maximum of 2.4 ng BDE-209/g lipid in Peru. ∑ $PBDE_6$ levels were in the range of background contamination below 2 ng/g lipid in five countries; the maximum ∑ $PBDE_6$ concentration was found in Antigua-Barbuda (13.8 ng ∑ $PBDE_6$/g lipid) with BDE-209 levels below the limit of quantification (<0.06 ng/g lipid) (Fig. 19).

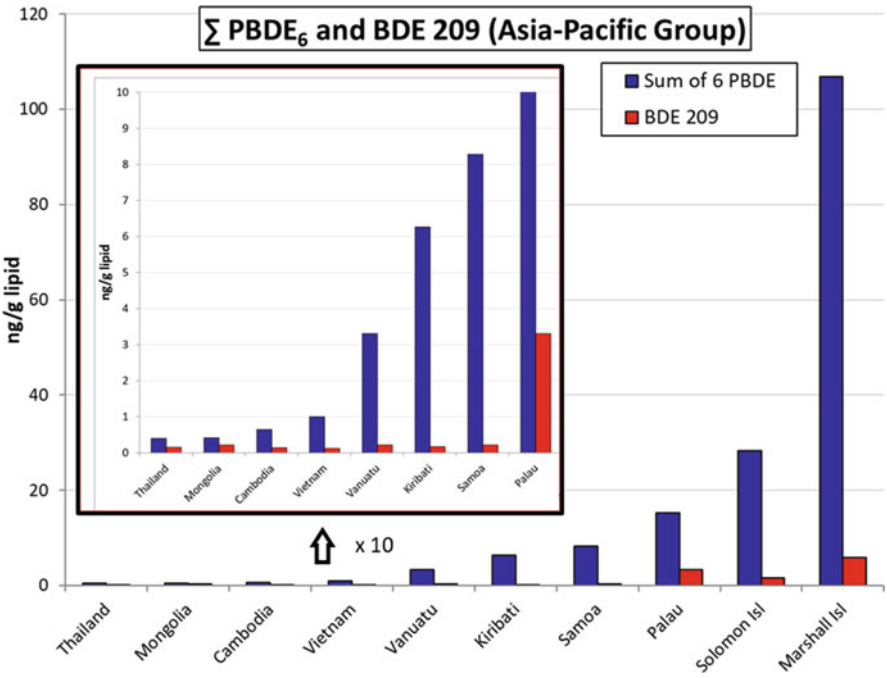

Fig. 18 Concentration of BDE-209 and ∑ $PBDE_6$ in human milk from countries in the Asia-Pacific Group for the period 2016–2019 (expressed as ng ∑ $PBDE_6$/g lipid and ng BDE-209/g lipid, respectively)

In nearly all ***European countries***, the BDE-209 concentrations were below 0.3 ng/g lipid. As maximum, 0.88 ng BDE-209/g lipid was found in Austria (41% of ∑ $PBDE_7$). The ∑ $PBDE_6$ levels were in the range of background contamination between 0.49 and 1.73 ng/g lipid (Fig. 20).

3.4 Congener Patterns

Within the group of the recommended six analytes, generally, BDE-47 showed the highest contribution to the ∑ $PBDE_6$ (49% as median of the five UN Regional Groups; range 16–73% in 134 pooled samples from 79 countries), followed by BDE-153 (21% as median of the five UN Regional Groups; range 4–55%), then BDE-99 (median 13%, range 7–35%) and BDE-100 (median 10%; range 6–20%). BDE-154 and BDE-175/183 contributed on average (as median) about 2% (for relevance of BDE-175 in this co-eluting pair and confirmation that BDE-175 is not present in technical products in quantifiable amounts, see discussion above in the introduction to Sect. 3 with reference to Konstantinov et al. 2011) (Fig. 21).

In the period 2016–2019, in addition to the six recommended analytes and BDE-209, 18 other congeners were included in the analysis of 40 samples from

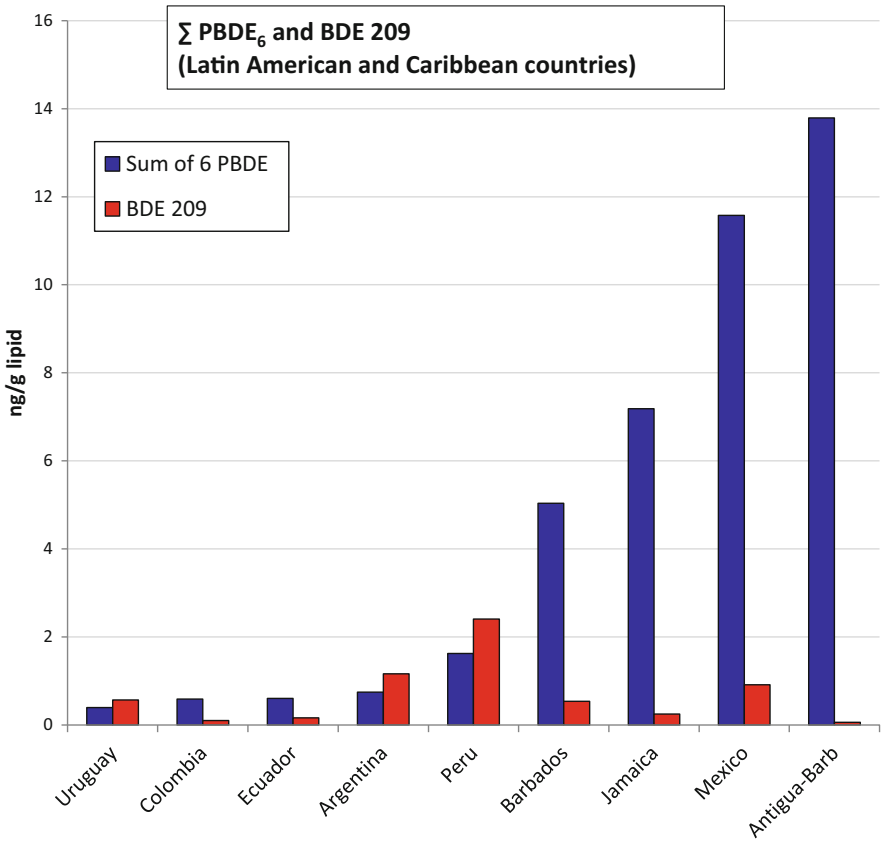

Fig. 19 Concentration of BDE-209 and $\sum$ $PBDE_6$ in human milk from countries in the Group of Latin American and Caribbean countries for the period 2016–2019 (expressed as ng $\sum$ $PBDE_6$/g lipid and ng BDE-209/g lipid, respectively)

39 countries to cover a broader range (BDE-15, BDE-17, BDE-28, BDE-49, BDE-66, BDE-75; BDE-77; BDE-85; BDE-119; BDE-126; BDE-138; BDE-190; BDE-196; BDE-197; BDE-203; BDE-206; BDE-207; BDE-208). Whereas BDE-28 and BDE-49 were included in EURL proficiency test, the other additional congeners were not included in any proficiency tests, and therefore are currently not validated with the same degree of quality control as the seven recommended analytes (Schächtele et al. 2023). However, results can give a first provisional indication whether other congeners might be of interest in addition to the seven congeners recommended by the guidance document (UNEP 2019). PBDE congeners up to hepta-brominated congeners were determined at relatively low levels in comparison with the recommended six analytes, if exceeding the range of the limit of quantification at all. However, the octa-BDE 197 and nona-BDE 206, 207, and 208 should be considered as possible recommended analytes in human milk in future activities: The contribution of these analytes to $\sum$ $PBDE_{25}$ was in the range of the six recommended

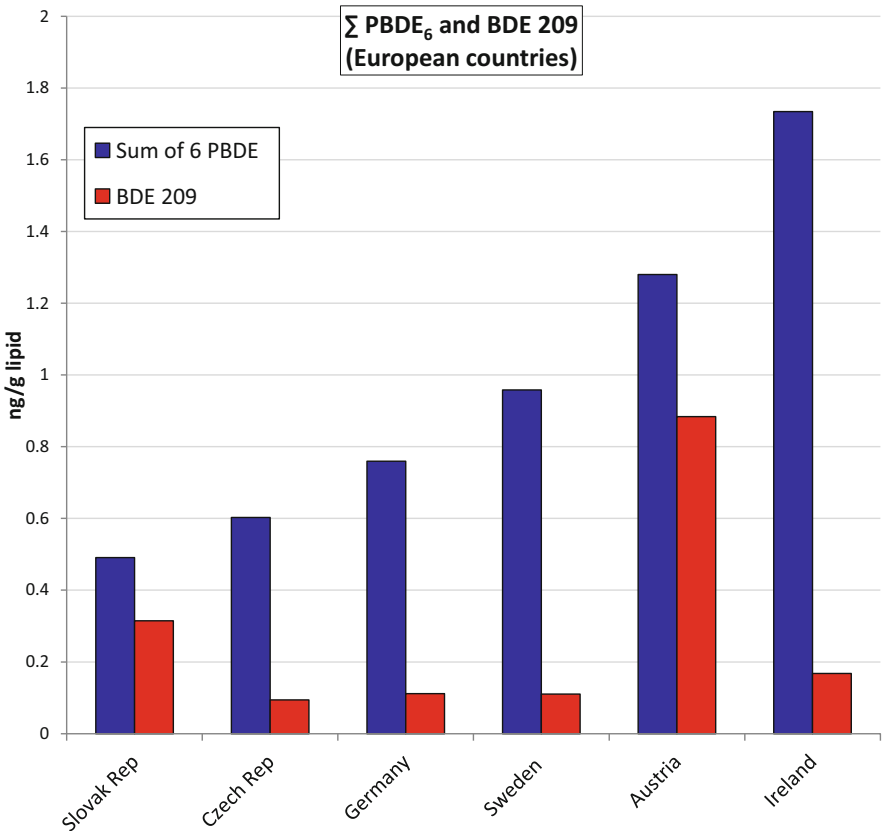

Fig. 20 Concentration of BDE-209 and ∑ $PBDE_6$ in human milk from European countries for the period 2016–2019 (expressed as ng ∑ $PBDE_6$/g lipid and ng BDE-209/g lipid, respectively)

analytes and BDE-209 (Fig. 22). These findings might be explained by suggestion of reductive debromination to nona- and octa-BDEs as the likely first step in the metabolism of BDE-209, with formation of BDE-206, BDE-207, and BDE-208 in the metabolic pathways as prerequisite for the formation of hydroxynona-BDE metabolites (EFSA 2011). The accumulation of higher brominated PBDEs in lactating cows was shown for BDE-196, BDE-197, and BDE-207, suggesting a metabolic debromination of BDE-209 to these BDEs (Kierkegaard et al. 2007). In human milk from China, BDE-209 a as congener considered to have less bioavailability was detected in about 50% of the samples at concentrations higher than that of other congeners. Other higher brominated congeners, including BDE-197 and BDE-207, were also prominent (Sudaryanto et al. 2008). Additionally, see the review on findings of POPs in human milk (Fürst 2023).

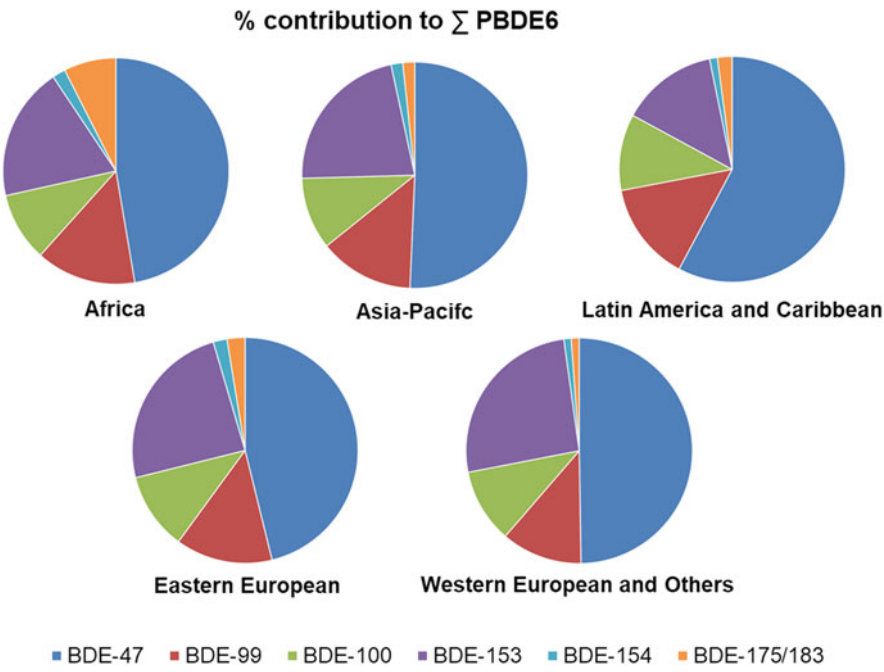

Fig. 21 Contribution (%) of 6 PBDE analytes to ∑ $PBDE_6$ in human milk from five UN Regional Groups (median of countries of the region)

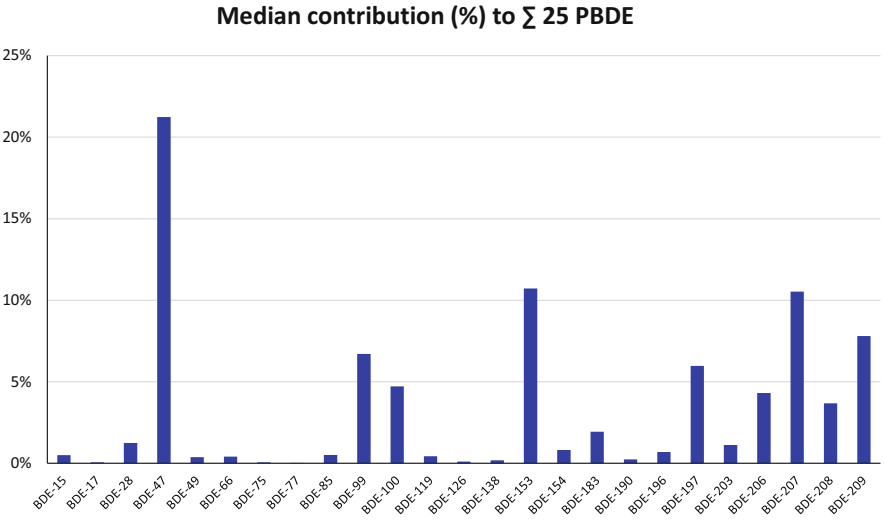

Fig. 22 Provisional assessment of the median contribution (%) of 25 di- to deca-BDE congeners to ∑ $PBDE_{25}$ in human milk in 39 countries from the five UN Regional Groups

4 Hexabromocyclododecanes (HBCDD)

Technical products of hexabromocyclododecane contain predominantly the three stereoisomers α-HBCDD, β-HBCDD, and γ-HBCDD, although other stereoisomers are theoretically possible. Whereas γ-HBCDD is the main compound in technical HBCDD, α-HBCDD is more persistent in the environment and biota, including humans (for literature review, see Fürst 2023 and Fång et al. 2015). This is possibly due to differences in persistence, or because of transformation processes (EFSA 2021).

The **α-HBCDD** levels of 102 pooled samples from 72 countries collected between 2006 and 2019 ranged between <0.1 and 15 ng/g lipid (median: 0.5 ng/g lipid; 90% of all results below 2 ng/g lipid). **β-HBCDD and γ-HBCDD** were in nearly all samples below the limit of quantification (LOQ for 90% of the samples: <0.1 ng/g lipid) or around the LOQ (max: 0.8 ng/g lipid). As a consequence, α-HBCDD is the predominant stereoisomer in human milk. Therefore, the sum parameter "**sum of the three stereoisomers**" is in close agreement with the α-HBCDD concentrations only: The lower bound "sum of the three stereoisomers" (where non-detects = 0) ranged between 0 and 15.7 ng/g lipid (median: 0.5 ng/g lipid; 90% of all results below 2.0 ng/g lipid) and the upper bound sum (where non-detects = limit of quantification) between 0.2 and 15.8 ng/g lipid (median: 0.7 ng/g lipid; 90% of all results below 2.4 ng/g lipid).

In all countries of the African Group (Fig. 23), the Asia-Pacific Group (Fig. 24) and the Group of Latin American and Caribbean Countries (Fig. 25), the **α-HBCDD** levels were in all surveys below 2 ng/g lipid. In the Eastern European Group (Fig. 26), five out of seven countries had α-HBCDD levels above 2 ng/g lipid in the 2012–2015 period, with a maximum of 15 ng/g lipid in Romania (2014). In Georgia and Moldova, the α-HBCDD increased from the 2008–2011 period (Georgia: 1.3 ng/g lipid; Moldova: 2.8 ng/g lipid) to the 2012–2015 period (Georgia: 4.0 ng/g lipid; Moldova: 8.0 ng/g lipid). In Czechia, the α-HBCDD concentration remained constant at 1.0 ng/g lipid between 2014 and 2019. In nearly all samples from countries of the Western European and Others Group (Fig. 27), the α-HBCDD concentration was below 2 ng/g lipid. The maximum level was from a sample from Austria (2016) that had a concentration of 5.6 ng/g lipid.

It is not possible to derive a temporal tendency: In all non-European countries with repeated participation, α-HBCDD concentrations tend to fluctuate at near background levels below 1–2 ng/g lipid seeming partly to decrease, partly to remain quite constant or partly to increase until the 2016–2019 period. In two Eastern European countries with repeated participation until the 2012–2015 period, temporal tendencies seemed to increase up to 8 ng/g lipid. As explained for PBDE, overall decrease rates as proposed for the ability of temporal studies to detect time trends for the effectiveness evaluation can be calculated for individual countries, but are of little relevance in cases, when background levels might have leveled out over time. More data for countries with levels above 2 ng α-HBCDD/g lipid would be necessary to derive reliable time trends for the comparably higher concentration range.

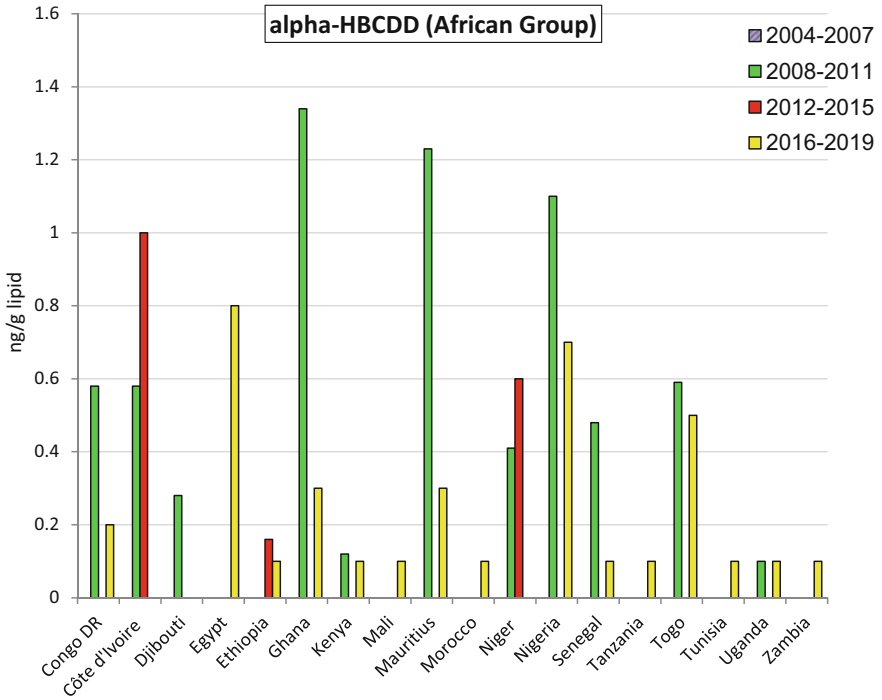

Fig. 23 Concentration of α-HBCDD in human milk from countries of the African Group (ng/g lipid)

5 Hexabromobiphenyl (PBB 153)

Hexabromobiphenyl comprises 42 isomers in one homolog group; PBB 153 is the recommended analyte (UNEP 2019). PBB 153 concentrations were below the limit of quantification (0.5 ng/g lipid) in 106 of 110 pooled samples from 69 countries. In four samples, low concentrations between 1.0 and 1.7 ng/g lipid were found.

6 Polybrominated Dibenzodioxins and Furans (PBDD/PBDF)

Thermal stress (waste combustion or accidental fires) of PBDE may result in formation of polybrominated dibenzodioxins and -furans (PBDD/PBDF congeners) or mixed brominated-chlorinated dibenzo-*p*-dioxins and dibenzofurans (PXDD/PXDF). Consequently, the use of PBDE as brominated flame retardants raised concern regarding environmental releases of brominated or mixed brominated-chlorinated dioxins and furans. Thus, for scientific reasons 38 samples from 28 countries collected between 2001 and 2009 were analyzed for PBDE (listed by the Stockholm Convention on POPs) as well as PBDD and PBDF (not listed by the

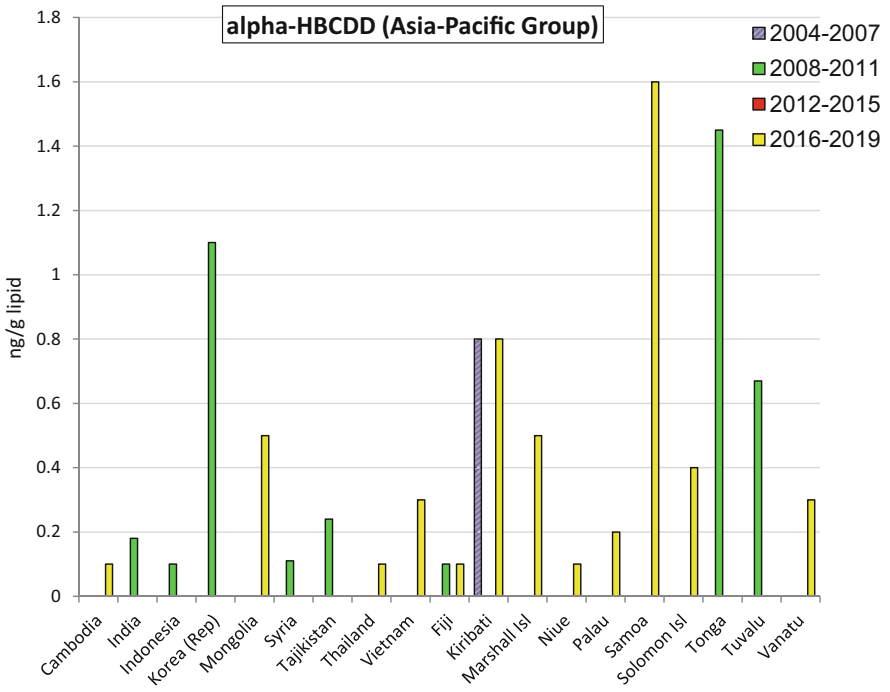

Fig. 24 Concentration of α-HBCDD in human milk from countries of the Asia-Pacific Group (ng/g lipid)

Convention). Moreover, polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (both listed by the Convention) were also measured in these human milk samples. The analyses of these brominated as well as chlorinated analogs provided the possibility to determine the contribution of PBDD and PBDF to the total amount of TEQ.

The same toxic equivalency factors (TEF) as derived by an WHO expert meeting in 2005 (WHO-TEFs [2005]) for PCDD and PCDF (Van den Berg et al. 2006) were also applied for PBDD/PBDF congeners (Kotz et al. 2005; Kotz 2006), as recommended as an interim approach (van den Berg et al. 2013). For more details of these TEF schemes and calculation of toxic equivalents as WHO-TEQ as "upper bound" (where non-detects = limit of quantification) and "lower bound" concentrations (where non-detects = 0), see Malisch et al. 2023b.

An overview of the results and the ratios between WHO-PBDD/PBDF-TEQ and WHO-PCDD/PCDF-TEQ are presented in Table 9. The differences between lower bound and upper bound WHO-PCDD/PCDF-TEQ concentrations are negligible (Malisch et al. 2023b). However, the considerably lower concentrations of the brominated compounds resulted in more cases of congeners below the limit of quantification, which causes higher upper bound values for WHO-PBDD/PBDF-TEQ and thus higher differences between lower and upper bound values. Then, for

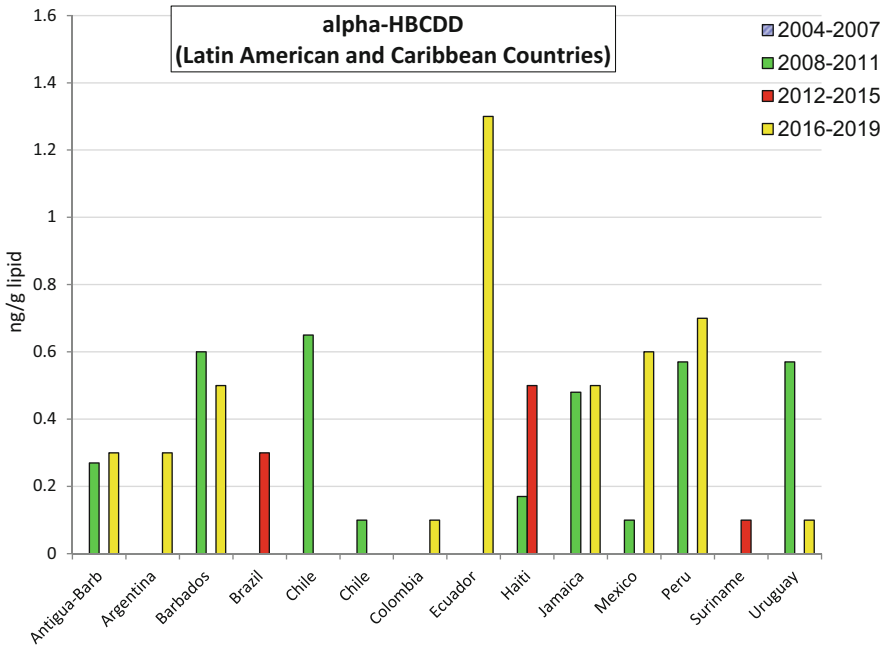

Fig. 25 Concentration of α-HBCDD in human milk from countries of the Group of Latin American and Caribbean Countries (ng/g lipid)

risk assessment, the application of the upper bound concentrations leads to an overestimation of the intake, the application of the lower bound concentrations to an underestimation of the intake. For these cases, the application of a "middle bound" TEQ concentration was recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Canady et al. 2002). Therefore, in addition also middle bound values are given for this parameter.

On average WHO-PBDD/PBDF-TEQ provided about 10% to the total amount of WHO-TEQ for both groups (chlorinated and brominated) of analogs combined. In view of this limited contribution of PBDD and PBDF to the total amount of WHO-TEQs, the risk–benefit analysis as done for the PCDD and PCDF (van den Berg et al. 2023) will only be influenced marginal and not change the main conclusions significantly.

Figure 28 compares levels of PBDD/PBDF and PCDD/PCDF (expressed as WHO-TEQ) with PBDE concentrations: Samples are sorted with $\sum$ $PBDE_6$ concentrations increasing from about 1 to 350 ng/g lipid, however, no correlations between PBDE and PBDD/PBDF or between PBDD/PBDF and PCDD/PCDF were found: The PBDD/PBDF levels in two human milk samples from the USA with highest PBDE concentrations (93 respectively 352 ng $\sum$ $PBDE_6$/g lipid; 0.58, respectively, 0.61 pg WHO-PBDD/PBDF-TEQ/g lipid, UB) were comparable to

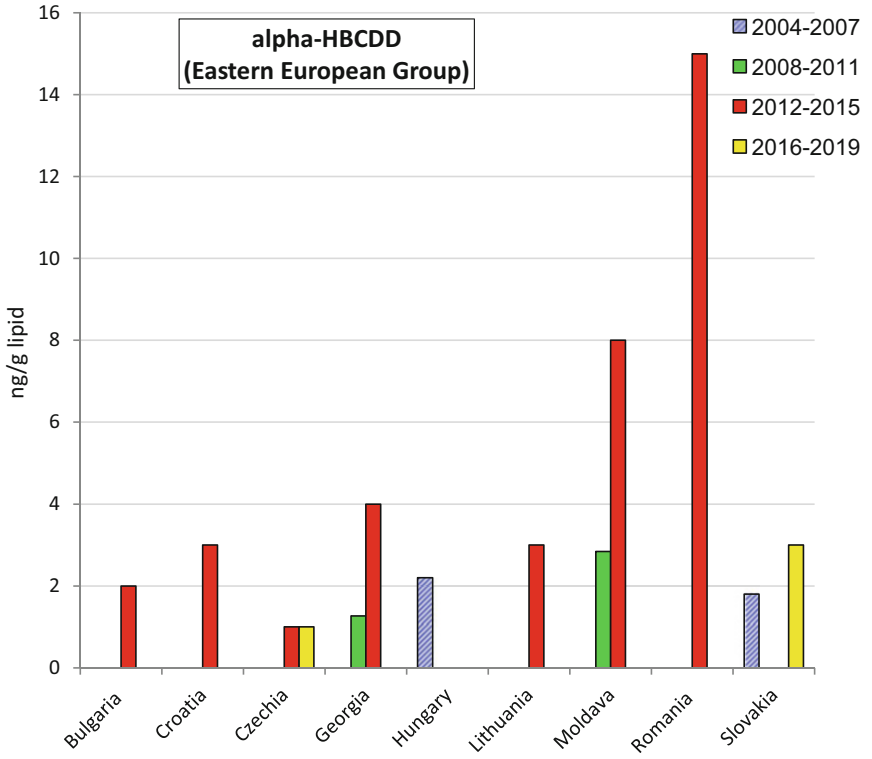

Fig. 26 Concentration of α-HBCDD in human milk from Eastern European countries (ng/g lipid)

the other countries within the same order of magnitude (median of all samples: 0.77 pg WHO-PBDD/PBDF-TEQ/g lipid, UB; range 0.12–1.58 pg/g), although the PBDE concentrations exceeded the levels in most other countries by two orders of magnitude.

The mixed brominated-chlorinated PXDD/PXDF pose a particular challenge: Of the theoretically possible 4600 congeners, there are 984 2,3,7,8-substituted compounds alone, among them the toxicologically most interesting tetra- and penta-substituted PXDD/PXDF congeners. Four 2,3,7,8-substituted TXDD (a total of 254 congeners) and eight TXDF (a total of 496 congeners) can theoretically occur. Among the penta-substituted ones, there are already twenty 2,3,7,8-PeXDD (420 in total) and forty PeXDF (480 in total). As only 2,3,7,8-substituted PCDD and PCDF are found in humans at the end of the food chain, it can be assumed that if PXDD/PXDF are detected in the human milk samples, they are only 2,3,7,8-substituted congeners. However, already the very limited number of standards restricts the specific determination of the individual 2,3,7,8-substituted congeners significantly, apart from analytical problems, e.g., chromatographic separation. Therefore, it was

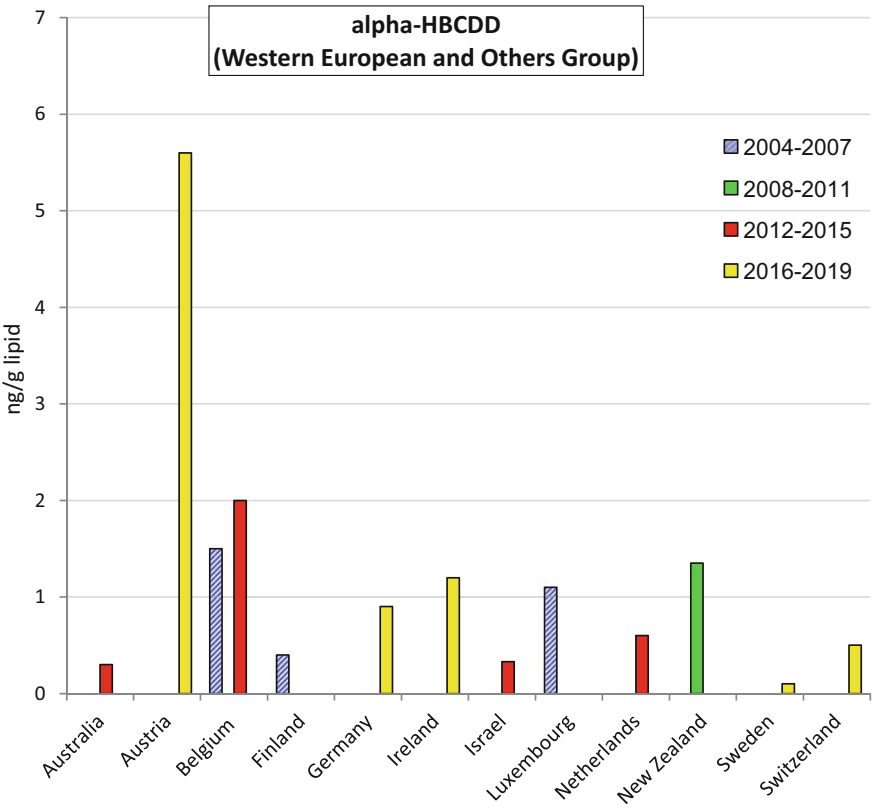

Fig. 27 Concentration of α-HBCDD in human milk from countries of the Western European and Others Group (ng/g lipid)

Table 9 WHO-TEQ values in pg/g lipid in 38 human milk samples from 28 countries for PBDD/PBDF, PCDD/PCDF, and their ratios

	WHO-PBDD/PBDF-TEQ			WHO-PCDD/PCDF-TEQ	Ratio WHO-PBDD/PBDF-TEQ to WHO-PCDD/PCDF-TEQ	
	LB	MB	UB		MB	UB
Min	0.00	0.09	0.12	2.48	0.02	0.02
Median	0.14	0.46	0.77	7.05	0.07	0.10
Mean	0.24	0.52	0.81	7.50	0.08	0.12
Max	0.71	1.01	1.58	16.25	0.19	0.27

LB, lower bound; MB, middle bound; UB, upper bound

checked whether tetra- and penta-substituted PXDD/PCDF could be detected at all in any of the human milk samples tested. However, they could not be detected in any sample. The limit of quantification of 0.05 pg/g fat for the TXDD/TXDF and PeXDD/PeXDF congeners was comparable to those of PBDD/PCDF. The

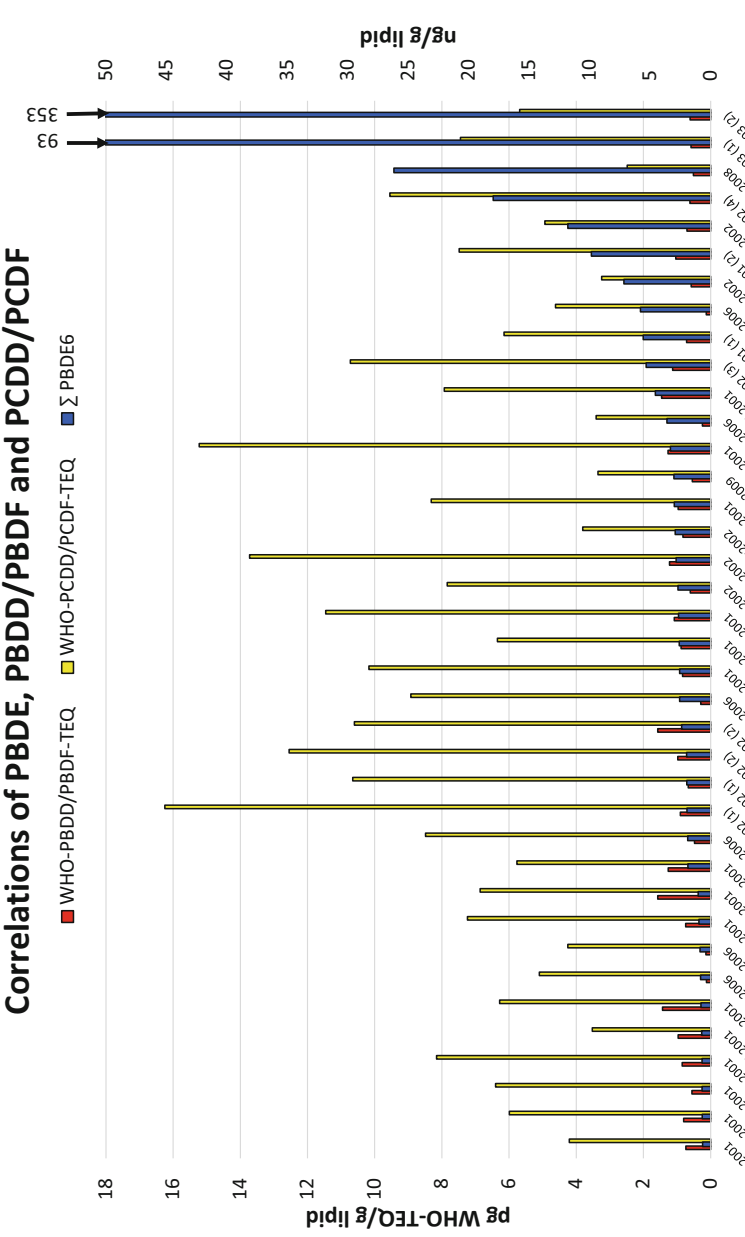

Fig. 28 Correlations between PBDE concentrations (ng $\sum$ $PBDE_6$/g lipid; samples sorted with increasing PBDE levels) with corresponding concentrations of WHO-PBDD/PBDDF-TEQ and WHO-PCDD/PCDF-TEQ (pg/g lipid, calculated using WHO-TEF [2005] as proposed by van den Berg et al. 2006, 2013, upper bound results)

calculation of the "WHO-PXDD/PXDF-TEQ" is not meaningful if too many congeners are not detectable (Kotz et al. 2005; Kotz 2006).

Acknowledgments This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region," funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk studies, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

Data analysis was supported by the RECETOX Research Infrastructure (No LM2018121) and the CETOCOEN EXCELLENCE Teaming Phase 2 project financed by the European Union's Horizon 2020 (No 857560) and the European Structural and Investment Funds: No CZ.02.1.01/0.0/0.0/17_043/0009632).

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure studies for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the WHO/UNEP Reference Laboratories, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the Reference Laboratories was an important aspect for the successful organization of these studies on a global level.

Hae Jung Yoon, Seongsoo Park, and Philippe Verger (WHO Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

We thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao, and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects during her time at the UNEP Economy Division, Chemicals and Health Branch.

We also thank the team at CVUA Freiburg for the performance of the analyses of the POPs presented in this publication.

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WHO- and UNEP-Coordinated Human Milk Studies 2000–2019: Findings of Chlorinated Paraffins

Kerstin Krätschmer, Walter Vetter, Jiří Kalina, and Rainer Malisch

Abstract

Chlorinated paraffins (CP) are complex mixtures of several million theoretically possible individual compounds. Contrary to medium-chain CP (MCCP, C_{14}–C_{17}) and long-chain CP (LCCP, C_{18}–C_{30}), the third sub-group investigated, short-chain chlorinated paraffins (SCCP, C_{10}–C_{13}), have been listed in 2017 in Annex A (Elimination) of the Stockholm Convention on Persistent Organic Pollutants. The concentrations of CP were determined in 84 nation-wide pooled human milk samples collected between 2009 and 2019 in 57 countries participating in exposure studies coordinated by the World Health Organization and the United Nations Environment Programme. Until 2015, only total CP content was determined. In light of on-going efforts to also add other CP groups to the Annexes of the Stockholm Convention and the glaring lack of data on the general background contamination worldwide, later analysis determined SCCP and MCCP and investigated the presence of LCCP (C_{18}–C_{20} only). CP were present in all 84 samples, ranging 8.7–700 ng/g lipid. A statistically significant increase rate

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_10

of total CP concentrations in human milk of 30% over 10 years was found on a global level, with a considerable variation between UN Regional Groups. Homologue group patterns indicated higher shares of MCCP and LCCP in industrialized countries and economically dependent areas. Compared to all other POPs analysed in the samples, the concentration of the sum of SCCP and MCCP was in most cases only surpassed by DDT, except European countries with high shares of PCB. Considering the ubiquitous presence of CP in humans worldwide, further investigation into toxicological effects and human exposure seems more pressing than ever, so that regulatory action may follow.

Keywords

Chlorinated paraffin · Human milk · Global WHO/UNEP studies · Pooled sampling · SCCP · MCCP · Homologue group pattern · Persistent organic pollutant

1 Introduction

Chlorinated paraffins (CP) are complex substance mixtures often produced by radical chlorination of mixed alkane feedstocks, resulting in millions of theoretically possible isomers within this substance class (Vetter et al. 2022). For regulation purposes, these substances are often classified and grouped by carbon chain length, discerning between short-chain (SCCP, C_{10}–C_{13}), medium-chain (MCCP, C_{14}–C_{17}), and long-chain CP (LCCP, $C_{>17}$) (PARCOM 1995; POPRC 2015). The U.S. Environmental Protection Agency additionally introduced a further subdivision between LCCP (C_{18}–C_{20}) and very long-chain CP (vLCCP, C_{21}–C_{30}), which is still rarely adapted in the literature (USEPA 2015, 2016). However, this subdivision is relevant from analytical point of view, because only LCCP (C_{18}–C_{20}) but not vLCCP (C_{21}–C_{30}) can be analysed by gas chromatography (Krätschmer et al. 2021b). Variations in chlorination degree and carbon chain length make the resulting products suitable for various industrial applications, some of which have a very high demand (USEPA 2009; Glüge et al. 2016; van Mourik et al. 2016).

1.1 Production and Environmental Fate

Large-scale industrial production and use of CP go back to the 1940s. As example, incidentally, the use as waterproofing and anti-mildew agent in uniforms and equipment led to the allocation of all produced CP in the United States of America to the US Army during World War II (Summary of War Regulations 1943). Today, CP are most commonly applied as plasticizers and flame retardants in a wide variety of products like PVC floors, paints, leather sealants or high-temperature lubricants (ECB 2005, 2008; Gallistl et al. 2018; Hahladakis et al. 2018). Although concrete production volumes are very hard to come by, it is generally accepted that the annual

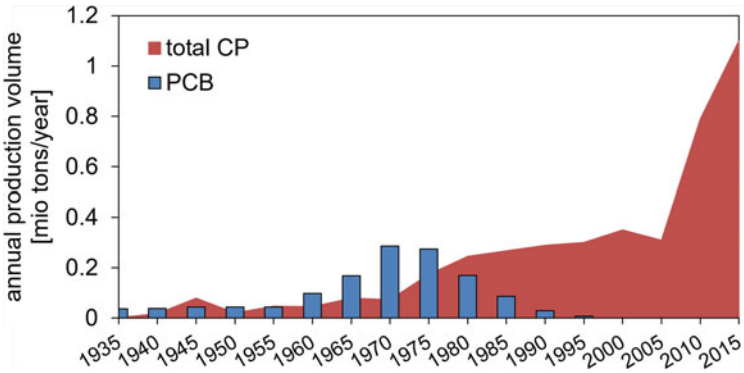

Fig. 1 Annual production volumes (metric million tons per year) of total CP (red) and PCB (blue columns) based on data reported by Breivik et al. (2002, 2007) and modelled by Glüge et al. (2016)

global production volume is extraordinarily high (Glüge et al. 2016). Coincidently, CP production surged at the same time when polychlorinated biphenyls (PCB) came under scrutiny and were later banned completely (OECD 1973; Breivik et al. 2007). Available data indicates a noticeable annual increase in the 1970s (Fig. 1) from less than 0.1 million tons/year (Muir et al. 2001) to an estimated 1.1 million tons/year in 2015 (Glüge et al. 2016). As a comparison, the estimated total production volume of PCB over six decades (1930–1993) was 1.0–1.5 million tons, with a peak annual production of <0.3 million tons/year reported in 1970 (Breivik et al. 2007; Stockholm Convention 2019).

Discussing production volumes of specifically SCCP and MCCP is practically impossible—China is currently thought to be the largest producer of CP having increased production from 0.6 million tons/year in 2007 (Fiedler 2010; Glüge et al. 2016) to 1.05 million tons/year in 2013 (POPRC 2015; van Mourik et al. 2016), but technical CP products are only defined by overall chlorination degree, not carbon chain length or CP group. Industry reports identified besides China also the Russian Federation, India, Japan, Brazil and several European countries as active CP producing countries, of which several (including China, India, the Russian Federation, Italy and France) have not ratified the Stockholm Convention decision to ban SCCP (ECHA 2008; POPRC 2010, 2015; Euro Chlor 2021). In contrast, at least SCCP production has been phased out in the USA (POPRC 2015), Canada (Environment and Climate Change Canada 2008), Sweden (Kemikalieinspektionen 2012, 2013), Japan (Tsunemi 2010) and the European Union (Commission Regulation (EU) No. 519/2012). Current reports and research indicate rapid growth of CP production in the Asia-Pacific (ASPAC) economic sector, with production volumes of primary CP applications like the production of polyvinyl chloride (PVC) reaching 1.3 million tons/year in India alone (Chaemfa et al. 2014; Zhang et al. 2017; Persistence Market Research 2021). Such uses as secondary plasticizers in soft plastics, paints and adhesives and flame retardants in many other products overshadow the direct use

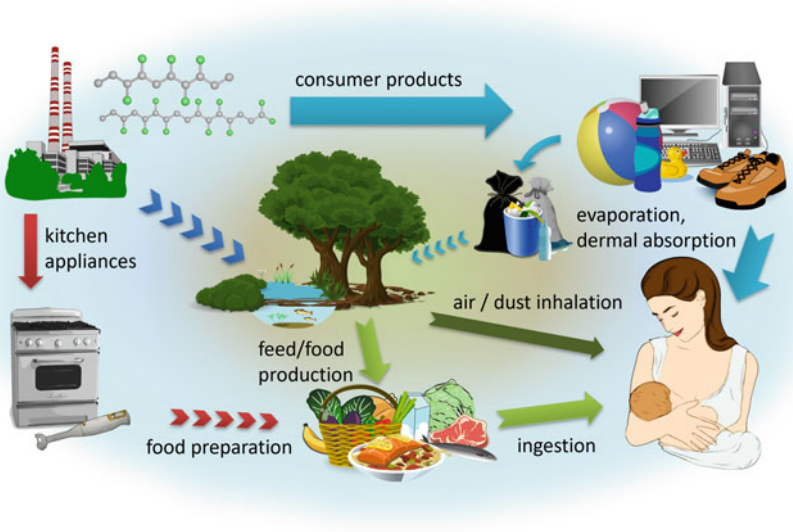

Fig. 2 Schematic representation of the main exposure ways to chlorinated paraffins (figure based on Krätschmer 2022)

of CP as lubricants and metalworking fluids (Tomy 2010; POPRC 2015; Zhang et al. 2017; Hahladakis et al. 2018).

Chlorinated paraffins can be released into the environment at every stage of their product life cycle (Fig. 2), starting with emissions from productions sites or in urban areas (Niu et al. 2021), abrasion during the use of recycled rubber floors (ECB 2005; Cao et al. 2018), to volatilization from waste storage, treatment or incineration (Nilsson et al. 2001; Feo et al. 2009; van Mourik et al. 2015; Brandsma et al. 2017; Matsukami and Kajiwara 2019; Matsukami et al. 2020). Not only the products, but also the recycling process of plastic itself is suspected of releasing CP (ECHA 2014; POPRC 2015). In the last decade, CP have been reported in the nature (air, sediment, water, biota) and domestic environment (indoor air, dust, household appliances, window coatings) and especially sediment is assumed to help distribute CP into benthic fauna and, consequently, into the food chain (Bayen et al. 2006; Feo et al. 2009; Wei et al. 2016; Glüge et al. 2018).

Besides CP found in (unprocessed) food, input from multiple sources during food processing, storage and preparation also has the potential to contribute to the total dietary intake of CP. CP have been found migrating from packaging materials into food simulants (Hahladakis et al. 2018; Wang et al. 2019), leaking from hand blenders (Yuan et al. 2017) and migrating into baked goods from oven isolation materials (Perkons et al. 2019). Using dishcloths (Gallistl et al. 2017) and fume hood filters (Bendig et al. 2013) as passive samplers and specifically targeting oiled hinges (Sprengel and Vetter 2021) and isolation coatings (Gallistl et al. 2018) of kitchen

appliances, a strong presence of SCCP and MCCP (in addition to several other polyhalogenated compounds) in German households could be shown.

Interestingly, studies of air samples found that emissions from CP productions plants only influence the immediate area, whereas CP levels in the urban environment seem to be caused by the numerous consumer products containing CP present in every household (Niu et al. 2021). Therefore, to decrease CP emissions, the use of CP in mainly plastic products needs to be decreased first. Because of this unique situation for such a class of high production volume chemicals, production bans and restrictions would serve more immediately to protect consumers than any maximum levels in food or the environment.

1.2 Toxicological Aspects

Regrettably, a large share of published toxicological studies on effects of SCCP and MCCP date back more than a decade and were often conducted using either complex technical mixtures or very few individual compounds for lack of alternatives (El-Sayed Ali and Legler 2010). In addition to that, analytical methods for CP were less reliable—any results should therefore be viewed as indicative rather than absolute. Available data suggest that CP exposure primarily targets the liver (Cooley et al. 2001; Du et al. 2019), kidneys (EFSA CONTAM Panel et al. 2020) and the thyroid and parathyroid glands (Cooley et al. 2001; Qiao et al. 2018), causing non-lethal effects in these organs. A feeding study in hens however did not report adverse effects of high CP doses, most likely caused by the quick excretion over several pathways (Ueberschär et al. 2007). Additionally, a study in Sprague-Dawley rats indicated that toxic effects of CP are inversely related to the alkyl chain length and directly to the CP′ mean degree of chlorination of CP (Geng et al. 2016). A physiologically-based pharmacokinetic (PBPK) model calculated for Sprague-Dawley rats and extrapolated to humans (Dong et al. 2019) found the half-life of SCCP with 5.2 years to be comparable to half-lives of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 3.2–11.3 years (Milbrath et al. 2009)) and perfluorooctanesulfonic acid (PFOS, 3.9–6.9 years (Olsen et al. 2007; Thompson et al. 2010)). The half-lives calculated for MCCP and LCCP however are much shorter (1.2 and 0.6 years, respectively). A recent review of available toxicological studies concluded that while it is reasonably sure that CP are absorbed in the gastro-intestinal tract and probably various other organs, the metabolic pathways and transformations are still poorly studied, leaving a wide gap in the knowledge on these compounds (Darnerud and Bergman 2022).

Based on reported neoplastic effects in male mice, the World Health Organization (WHO) recommended a tolerable daily intake (TDI) of 11 μg SCCP per kg bodyweight and day in 1996 (WHO et al. 1996). This TDI value already included a security factor of 1000. The European Food Safety Authority EFSA however stated in 2020 in their Scientific Opinion that available toxicological data is insufficient for concluding on a TDI for any of the CP groups (EFSA CONTAM Panel et al. 2020). Instead, EFSA modelled a benchmark dose lower confidence limit 10% value

($BMDL_{10}$), representing the lowest dose that causes no more than 10% incidences of the chosen limiting effect (SCCP: increased liver weight, MCCP: nephritis) in rats (EFSA CONTAM Panel et al. 2020). The $BMDL_{10}$ have been set to 2.3 mg/kg bodyweight/day (SCCP) and 36 mg/kg bodyweight/day (MCCP) with a margin of exposure of 1000, although there have been protests in the scientific community stating that these values are too high, especially for MCCP (Zellmer et al. 2020). No limiting value of any kind could be established for LCCP, although kidneys were identified as likely target organs (EFSA CONTAM Panel et al. 2020).

Concerning infant's exposure to POPs and CP in particular, studies suggest that POPs found in human milk are likely to be present in the infant's blood as well (Chakraborty and Das 2016). Besides this lactational exposure, a study comparing paired maternal and umbilical cord serum samples was able to show evidence of placental transfer to the infant in utero (Qiao et al. 2018). This emphasizes the need for conservative and updated risk assessments in the interest of protecting vulnerable population groups and consequently the need for more occurrence and toxicological data.

1.3 Classification as POP and Regulatory Situation

Due to the difference in the regulatory status, it is important to distinguish between SCCP (listed as POPs) and MCCP (unregulated, candidate POPs) when discussing CP results. Both SCCP and MCCP have been under scrutiny for their persistent (Muir et al. 2001; ECHA 2008), bioaccumulative (Fisk et al. 2000; Houde et al. 2008; Yuan et al. 2019) and toxic (Cooley et al. 2001; El-Sayed Ali and Legler 2010; Geng et al. 2016) properties, but only production and worldwide use of SCCP was severely restricted by the parties that ratified this part of the United Nations' (UN) Stockholm Convention in 2017 (Conference of the Parties of the Stockholm Convention 2017).

Shortly after classifying SCCP as POPs, the need for more occurrence data and toxicological studies to expand on existing data (Fisk et al. 2000; ECB 2005; Thompson and Vaughan 2014; Yuan et al. 2019) came into focus of research groups and official control bodies. Especially since MCCP but not SCCP are classified as 'may cause harm to breastfed children' under the harmonized classification of the EU Classification, Labelling and Packaging (CLP) Regulation (ECHA 2019), an in-depth assessment of potential health risks of MCCP seems prudent (Swedish Chemicals Agency 2018; EFSA CONTAM Panel et al. 2020; Zellmer et al. 2020).

Despite EFSA's findings seemingly indicating low risk from MCCP, the European Chemicals Agency ECHA declared them candidate Substances of Very High Concern (SVHC) in 2021 with proven toxic, persistent and bioaccumulative properties (ECHA 2021a, b). Upon conclusion of the assessment period, a restriction of MCCP to 0.1% in products and other chemicals is expected within the European Union (EU). Additionally, MCCP are scheduled to be assessed for addition to the Annexes of the Stockholm Convention starting early 2022 (Stockholm Convention 2021).

1.4 CP as Target Analytes in the WHO/UNEP-Coordinated Human Milk Studies on Persistent Organic Pollutants

Analysing human tissue allows for monitoring of the total exposure levels of target contaminants in the population. Of the possible tissue samples, human milk presents the preferable option as the sampling process is non-invasive and yields a much higher lipid content than blood serum.

The WHO and the United Nations Environment Programme (UNEP) performed seven global human exposure studies for certain persistent organic pollutants (POPs) between 1987 and 2019. Initially, these studies were conducted by WHO for polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). After a sufficient number of countries ratified the Stockholm Convention on POPs in 2004, WHO and UNEP agreed to collaborate in joint studies to support the implementation of the convention. The number of POPs initially covered by the convention was expanded considerably since its adoption (UNEP 2020). SCCP were added in 2017 to Annex A to the Convention for elimination of production and use of chemicals. The Article 16 requires the periodic evaluation of the effectiveness of the convention in reducing emissions of POPs. One of the pillars of this evaluation is to be based on comparable and consistent monitoring data on the presence of POPs in the environment and in humans. Therefore, four more rounds were organized by WHO and UNEP between 2005 and 2019.

As such, human milk samples are often used to report occurrence levels of lipophilic contaminants such as polychlorinated biphenyls (PCB) (Mamontova et al. 2017; Müller et al. 2017; Bawa et al. 2018), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) (Abballe et al. 2008; Fång et al. 2015), brominated flame retardants (BFR) (Antignac et al. 2016), organochlorine pesticides (OCP) (Al Antary et al. 2017; Polanco Rodríguez et al. 2017; Bawa et al. 2018; Chen et al. 2018) and CP (Cao et al. 2017; Xia et al. 2017a, b; Yang et al. 2018), see also review in Part I of this book (Fürst 2023). Cross-comparisons of these studies are however exceedingly difficult, as the necessary ethical clearances and budget constraints seem to result in small studies with mixed parity of the mothers, only rarely screening for contamination sources and mixtures of individual, city-wide grouped or regionally grouped samples. Especially individual samples often show high variability and hinder comparisons on an international level (Krätschmer et al. 2021a).

The sampling protocol implemented by the WHO and UNEP in their human milk studies however results in cost-effective nation-wide pools which are considered to represent countries or subpopulations at the time of sampling (Malisch et al. 2023c). The gradual addition of new persistent organic pollutants to the analysis portfolio of these studies since entry into force of the Stockholm Convention in 2004 (Stockholm Convention 2013) led to the addition of SCCP as optional parameter in 2012 in these studies.

In accordance with the implementation of the Global Monitoring Plan (GMP), parties report through one of the five UN Regional Groups (Malisch et al. 2023c).

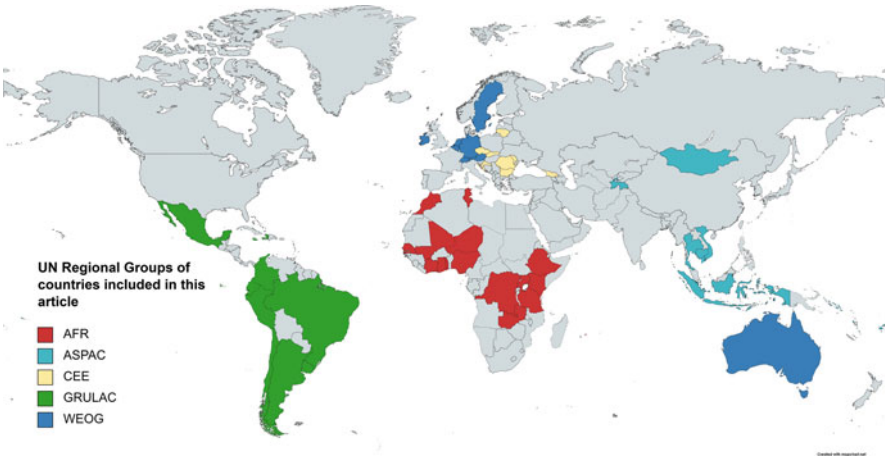

Fig. 3 Overview of countries discussed in this article, with assinged UN Regional Groups. AFR, Africa; ASPAC, Asia-Pacific Group; CEE, Central and Eastern Europe; GRULAC, Group of Latin American and Caribbean Countries; WEOG, Western European and Others Group (including Australia)

Therefore, countries are classified according to one of the following five geopolitical groups (DGACM 2019) for the purposes of this article: the African Group (AFR), the Asia-Pacific Group (ASPAC), the Group of Latin American and Caribbean Countries (GRULAC), the Central and Eastern Europe (CEE) and the Western European and Others Group (WEOG). Notably, Australia is a member of the WEOG instead of the geographically closer ASPAC group (Fig. 3).

The method used for analysis of samples collected between 2009 and 2015 at the State Institute for Chemical and Veterinary Analysis (CVUA) Freiburg produced results which can be discussed as total CP values only (Krätschmer and Schächtele 2019). With regard to plans to add specifically SCCP (and not MCCP or LCCP) to the listed compounds, a new method using high-resolution mass spectrometry with Orbitrap technology was established and applied to the human milk samples starting 2016. This method allowed for the distinction between SCCP and MCCP as well as qualitative detection of some LCCP (Mézière et al. 2020; Krätschmer et al. 2021a, b; Schächtele et al. 2023); it was used also for re-analysis of samples arriving after 2011, if sufficient sample amount was left. Suitability of this method was shown through participation in several interlaboratory studies and proficiency tests on food and biota samples containing SCCP and MCCP (Krätschmer and Schächtele 2019; Schächtele et al. 2023).

All substance-specific data were deposited at the Global Monitoring Plan Data Warehouse (GMP DWH), which can be publicly accessed. This serves as the source of information for the regional and global reports of the GMP and for the evaluation of the effectiveness of the convention to eliminate or reduce emissions of selected POPs (Global Monitoring Plan Data Warehouse 2020).

2 Materials and Methods

2.1 Sample Collection

The detailed study design, rationale and sample collection procedure are described elsewhere (Krätschmer et al. 2021b; Schächtele et al. 2023). To summarize, national coordinators in each participating country organized representative sampling campaigns, with the eligibility criteria applied to the mothers including willingness to breastfeed, age of the mother, primiparity, expectation of a singleton, healthy pregnancy, a minimum time of 10 years residency in the area and the absence of known POP hotspots near the place of residence. Samples were collected 3–8 weeks after birth with informed consent of the mothers, of which 25 mL was analysed locally for basic POPs (e.g., indicator PCB or pesticides). The rest of each sample from at least 50 mothers was added to one pooled milk sample per country and shipped frozen to CVUA Freiburg (Germany) and Örebro University (Sweden) for analysis. Lower numbers of contributors (at least 25 mothers) to the pooled samples were accepted in the case of small countries. The present report solely includes results produced in Freiburg.

2.2 Sample Preparation

Sample preparation of the pooled samples was performed as described elsewhere (Krätschmer et al. 2019, 2021a). In brief, 50 g of the hand warm, homogenized sample was treated with cooled centrifugation (4 °C, 3000 rpm, 10 min) in baked-out glass centrifuge tubes to separate the cream from the hydrogenous phase. After adding the recovery standard ($^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane, Cambridge Isotope Laboratories, Tewksbury, MA, USA), the cream was then dried by grinding with sodium sulphate until a powdery consistency was reached. Cold extraction with dichloromethane/n-hexane (1:1, v/v) was performed and the filtered solvent evaporated to dryness.

Further sample clean-up was performed using glass column chromatography using an acidified silica column and fractionation on a Florisil column (magnesium silicate primed with 1.5% water). The second fraction containing CP was then concentrated before the addition of the syringe standard ε-hexachlorocyclohexane (ε-HCH, Dr. Ehrenstorfer, Augsburg, Germany) for analysis.

2.3 Measurement of SCCP and MCCP

Given the major developments in this field of analysis in recent years, the method applied to human milk samples at CVUA Freiburg has also changed over the years. Until 2016, CP samples were analysed by GC-EI-MS/MS (Reth et al. 2005), using the average results of three monitored mass transitions (*m/z* 102 → 67, *m/z* 102 → 65, *m/z* 91 → 53). This allowed for a reliable and comparable quantification of the

total CP amount in the sample, but not for a distinction between SCCP and MCCP. The new method introduced 2017 used GC-ECNI-Orbitrap-HRMS, with a high enough mass resolution and full scan mode able to discern between SCCP and MCCP on the homologue group level (Krätschmer et al. 2018).

While high-resolution mass spectrometry often allows for quantification of the full spectrum of homologue groups in order to quantify CP of a certain alkyl chain length, a reduction of the number of mass traces to be monitored would also help speed up establishing CP analysis more widely in environmental and food laboratories. It needs to be pointed out though that the chlorination patterns shown here were originally determined by GC-ECNI-HRMS and later corrected for their known shift toward higher chlorination degrees with a set of correction factors (Mézière et al. 2020).

2.4 Quality Control

Each sample batch additionally included a procedural blank (sodium sulphate prepared like a sample) as well as different quality control (QC) samples. To match the pooled human milk samples of the present study, these QC samples consisted of raw cow's milk with and without the fortification with SCCP and MCCP standards on different levels. Recoveries ranged 83–110% (SCCP) and 84–119% (MCCP) for the GC-ECNI-Orbitrap method and 84–101% (CP) for the GC-EI-MS/MS method, respectively. Procedural blanks were 0.166 ± 0.023 ng/g sample CP for the samples from 2012 to 2017 and 0.098 ± 0.016 ng/g sample SCCP and 0.046 ± 0.038 ng/g sample MCCP for samples from 2017 to 2021. Limits of quantification (LOQs) were determined based on validation studies and the procedural blank levels.

As reported elsewhere, LOQs for human milk samples using the GC-ECNI-Orbitrap-HRMS method were 7.1 ng/g lipid SCCP and 12 ng/g lipid MCCP (Krätschmer et al. 2021a). All samples investigated with this method were above the LOQ. The LOQ for human samples using the GC-EI-MS/MS method was 38 ng/g lipid CP. Total CP results for three of the 27 pooled samples analysed using this method were below LOQ. More detailed description of the initial and ongoing validation process, interlaboratory exercise results and the different quantification approaches can be found in a different part of this book (Schächtele et al. 2023).

2.5 Assessment of Temporal Trends

General temporal trends were assessed for total CP content (all 84 samples, collected 2009–2019 in 57 countries) and for SCCP and MCCP concentrations (57 samples/countries collected 2015–2019; GC-Orbitrap-HRMS). Evaluation was based on the non-parametric linear Theil-Sen trend estimator (Sen 1968; Theil 1992) in order to address that exponential trends were expected (as commonly observed in cases after stop of production and application of a chemical rather than unrealistic linear trends

(Sharma et al. 2021)). The R package 'Median-based Linear Models (mblm)' (Komsta 2013) was used for this regression.

Trends were positively assigned if the trend test (significance of the Theil-Sen estimator) was positive on 95% confidence level of significance (i.e., p-values $<$ 0.05). Simulations showed that the Theil-Sen p-values are never $<$0.05 for less than 5 data points. Yet, the required five data points were not available for any country. This prevented that statistically significant temporal trends could be derived for individual countries. However, pooling of data in regions allowed us to investigate statistically significant time trends for several UN Regional Groups and at the global level (Sect. 3.1).

2.6 Grouping of Countries into UN Regional Groups

For the purpose of this article, participating countries are not grouped by geographical aspects, by according to the United Nations Regional Groups, although including the non-member state Kiribati in the Asia-Pacific Group.

3 Results for Samples Collected 2009–2019

3.1 Global Overview of Sum of CP Levels

As mentioned in the previous section, samples of the 2009–2011 period and some samples of the 2012–2013 period were only analysed on the sum of CP. Only a few samples collected in 2012–2013 and all samples arriving after 2014 were (re-) analysed on both SCCP and MCCP and on homologue group patterns. For a better comparison with older samples, the new samples were also discussed by means of their sum of CP values.

Remarkably, average CP levels in the Asia-Pacific Group (ASPAC) and Central and Eastern Europe (CEE) were lower in 2009–2014 compared to the period 2015–2019 (Fig. 4). By contrast, average CP levels in the Western European and Others Group (WEOG) seem to have decreased in the more recent time period, with a comparatively close grouping of the results. The median results of the African Group (AFR) and the Group of Latin America and Caribbean Countries (GRULAC) were very similar in both time spans so that no trend could be observed. It needs to be pointed out though that two data points are by no means sufficient to indicate an overall time trend for a region or country. Still, an overall indication of increasing CP levels in the background contamination of human milk in two out of five UN Regional Groups is cause for concern.

Further evaluation of temporal trends of the CP levels in the five UN Regional Groups was carried out with the Theil-Sen method to derive changes over a 1- and 10-year period (Fig. 5). Again, no significant changes of CP levels were observed in the AFR and GRULAC groups as a whole, while a decrease by 63% was calculated in the WEOG group over 10 years ($p = 0.001$). In contrast, in the ASPAC and CEE

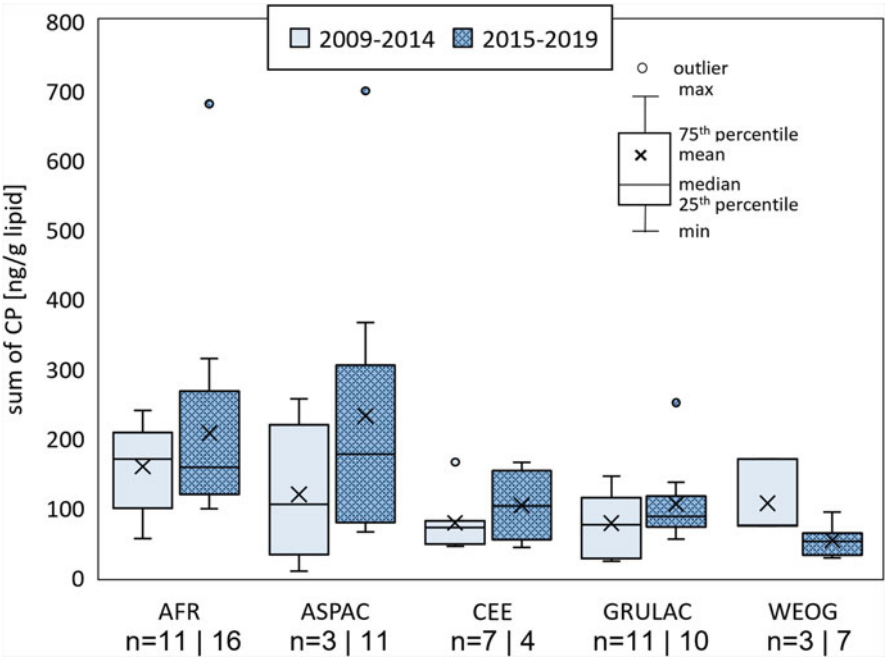

Fig. 4 Range of sum of CP determined in pooled human milk samples from the 2009 to 2019 period of WHO/UNEP-coordinated human milk studies. AFR, Africa; ASPAC, Asia-Pacific Group; CEE, Central and Eastern Europe; GRULAC, Group of Latin American and Caribbean Countries; WEOG, Western European and Others Group (including Australia)

groups CP increased by up to 200% over the decade ($p < 0.010$). The 10-year-trend worldwide, based on all 84 results of 57 countries covering the period between 2009 and 2019 indicated an increase of total CP in human milk by 30% ($p < 0.001$) (Table 1, Fig. 5).

Whereas for the assessment of temporal trends (over a decade) of total CP concentrations could be based on a sufficient number of samples for nearly all UN Regional Groups and globally, the available SCCP and MCCP data covered only a much shorter periods in most groups (AFR: 2015–2019; ASPAC: 2018–2019; GRULAC: 2012–2019; EEC: 2014–2019; WEOG: 2013–2019). Therefore, temporal trends in individual UN Regional Groups could not be examined. Yet, on a global level (using all available data) indicated an increasing trend for both MCCP and especially SCCP (Table 2), although only the observation for SCCP is statistically significant ($p < 0.001$).

This overall increasing trend was in line with predicted increase of the CP production (Glüge et al. 2016) and diametrically opposed to the overall decreasing trends for most of the other POPs monitored within the UNEP human milk survey (Malisch et al. 2023a, d).

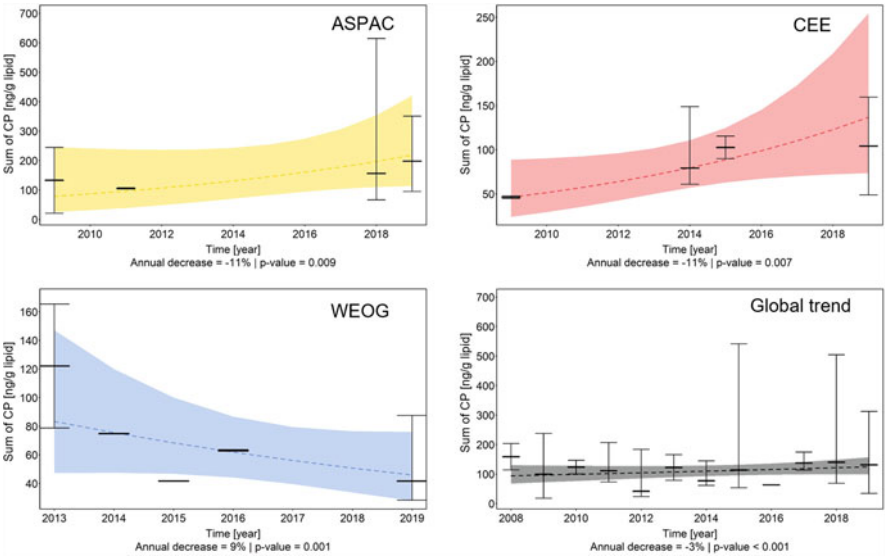

Fig. 5 Temporal trends of the sum of CP concentrations in pooled human milk samples in ASPAC, CEE and WEOG UN Regional Groups and globally (ng total CP/g lipid). Decrease rates and uncertainty were determined using the Theil-Sen method. Negative decrease rates are to be read as increase in that time period

Table 1 Annual and decennial trends for the sum of CP in human milk determined using the Theil-Sen method. Negative decreases are to be read as increase

		Decrease overall [%]		
UN Regional Group	n	Annual	Decennial	*p*-value overall
Africa	27	–0.6	–6.3	0.663
ASPAC	15	–11	–179	0.009
GRULAC	21	1.0	9.5	0.737
CEE	11	–12	–197	0.007
WEOG	10	9.4	63	0.001
global trend	84	–2.6	–29	<0.001

n, number of samples

Table 2 Annual and decennial trends for SCCP and MCCP in human milk worldwide determined using the Theil-Sen method. Negative decreases are to be read as increase

		Decrease overall [%]		
Parameter	n	Annual	Decennial	*p*-value overall
SCCP	57	–8.9	–134	<0.001
MCCP	57	–4.3	–53	0.237

n, number of samples

3.2 A Closer Look by Geographical Area

Within each geographical area, a wide range of CP levels was found (Figs. 6 and 7). Most notably, the samples from Mongolia (2018, 700 ng/g lipid CP) and Niger (2015, 680 ng/g lipid CP) were higher than other samples from their regional groups. However, an earlier sample from Niger (2011, 240 ng/g lipid CP) was markedly lower in its CP levels, indicating a considerable increase in comparison with the 2015 pooled sample. The sum of CP increased between two sampling periods by more than a factor of 2 also in Mauritius, Czech Republic, Peru and Uruguay.

On average, African and Asia/Pacific pooled samples had the highest combined SCCP and MCCP content of the five UN Regional Groups: with a median of 160 ng/g lipid each and mean results of 190 ng/g lipid (AFR) and 200 ng/g lipid (ASPAC), respectively, they surpassed mean and median levels of the other three groups by a factor of 2–3. Looking at the standard deviation of samples from the same UN region, AFR and ASPAC again surpass the other Regional Groups with 120 and 170 ng/g lipid or 63–84%, respectively. These high standard deviations are mostly caused by a few countries with remarkably high CP levels, but also based on a higher general variation between the different countries.

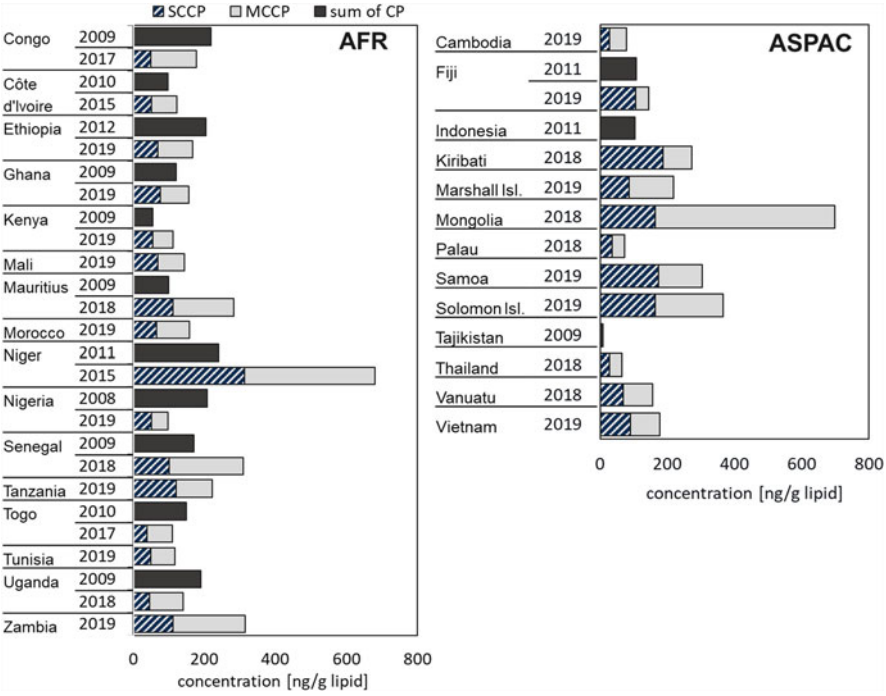

Fig. 6 SCCP, MCCP and sum of CP amounts for pooled country samples of human milk from member states of the African Group (AFR) and the Asia-Pacific Group (ASPAC)

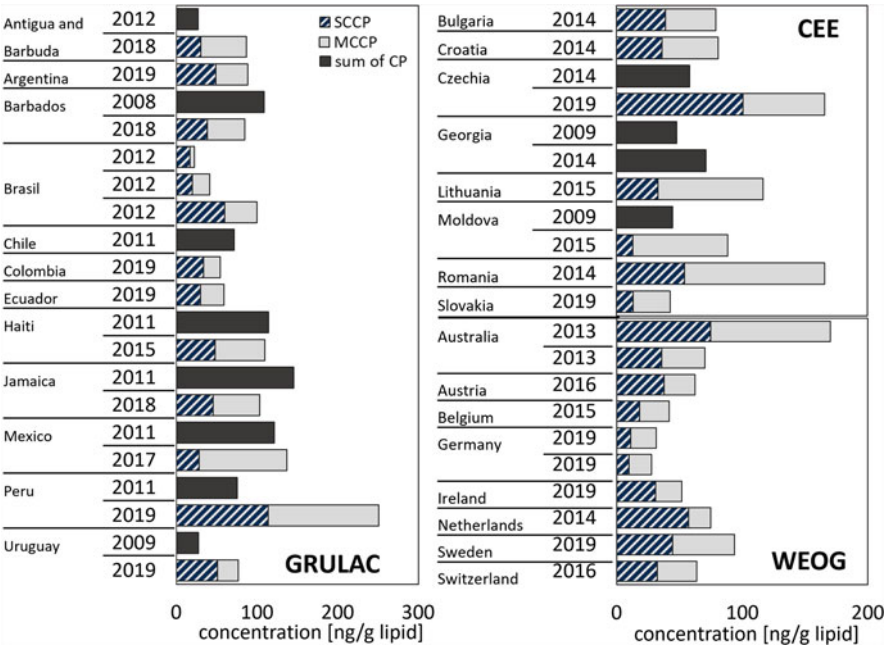

Fig. 7 SCCP, MCCP and sum of CP results for pooled country samples of human milk from member states of the Central and Eastern Europe (CEE), Western European and Others Group (WEOG) and Group of Latin American and Caribbean Countries (GRULAC). Brazil submitted three samples from different regions in the same year, see Sect. 3.2

Interestingly, variations between country samples in Europe, Western countries and South/Latin America were much smaller in absolute numbers with only ~40–50 ng/g lipid, but comparable to the African groups' relative standard deviation with a range of 51–59%. This is especially notable since WEOG also includes Australia as a geographically separated member, drastically widening the area covered by the otherwise very continent- or region-specific groupings. However, looking solely at Pacific Island States within the ASPAC group, a much lower standard deviation and more uniform distribution of SCCP and MCCP was observed, with the exception of Palau (2018). The standard deviation of the total CP amount for these countries was 87 ng/g lipid or 36%. While the overall CP levels seem more comparable in this area, available specific data on SCCP and MCCP levels show a higher variation of MCCP between these countries (56 ng/g lipid or 50%).

Unfortunately, the available population data on these pooled samples does not allow for more specific investigations of reasons for the observed differences between different countries in the same UN Regional Group. Especially the considerable number and diffuse nature of possible contamination sources causing exposure to CP means that despite careful planning and canvassing participants for the human milk studies in order to avoid known hotspots for other POPs, some

individuals with markedly higher CP exposure levels might have been included as one of the 25–50 donors contributing to the pooled samples. Such outliers might lead to a bias in the pooled sample, more so if the overall observed concentrations are on the lower end of the concentration range (Gewurtz et al. 2011).

Even without such a scenario, differences in consumption behaviour, underlying medical conditions, living environment or travel habits can lead to variations in the individual and pooled human milk samples. For example, the presence of many plastic (i.e., polyvinyl chloride, PVC) consumer products and building materials might cause higher daily CP exposure, for example through transfer into food from packaging material or through evaporation from electronic equipment or flooring (Schinkel et al. 2019; McGrath et al. 2021). Studies also suggest that rapid weight loss or (potentially undiagnosed) underlying medical conditions like type-2 diabetes mellitus can lead to an increased release of POPs into the blood circulation instead of accumulation in fatty tissue (e.g., Jansen et al. 2017; Berg et al. 2021), although no data on CP are available.

The larger differences in results observed between pooled samples from the same country and sampling year, i.e. three pooled samples from Brazil (2012), and two pooled samples from Australia (2013), but not between two pooled samples from Germany (2019), however are caused by different population groups and/or regions being sampled. The direct comparison of country-specific results also shows that samples belonging to CEE, GRULAC and WEOG were in most cases below 150 ng/g lipid CP, while AFR and ASPAC samples surpassed that mark in 15 of 27 (AFR) and in 8 of 15 (ASPAC) cases, respectively.

The comparison between Western and Eastern European countries is interesting in the context of the EU—even though most of the countries shown as part of CEE and WEOG in Fig. 7 have been subject to the same POPs regulations since the early 2010s (Commission Regulation (EU) No. 519/2012), there is a distinction between most Western European countries with equal or higher shares of SCCP and most Eastern European countries with equal or higher shares of MCCP.

As likely reason for these differences between countries that are in close proximity to each other, the locations of CP production sites might be considered, which included Germany, France, Italy, Spain, Slovakia, Romania and the United Kingdom (ECHA 2008; Euro Chlor 2021). However, studies of air samples found that emissions from CP productions plants only influence the immediate area, whereas CP levels in the urban environment seem to be caused by the numerous consumer products containing CP present in every household (Niu et al. 2021). Therefore, the wide use of CP, e.g., in mainly plastic products needs to be considered, as well. With the exception of Germany, available pooled human milk samples from these countries seem to have a slight dominance of MCCP, which fits with the predominant production and use of MCCP and LCCP even before the EU ban of SCCP production in 2012 (European Commission 2012) combined with the long product life of SCCP put on the market before 2012.

Regarding the relation between SCCP and MCCP, MCCP levels at least equalled SCCP levels in most pooled country samples of all UN Regional Groups, contributing 24–85% to the total CP levels reported here (Fig. 8). In 36 of the

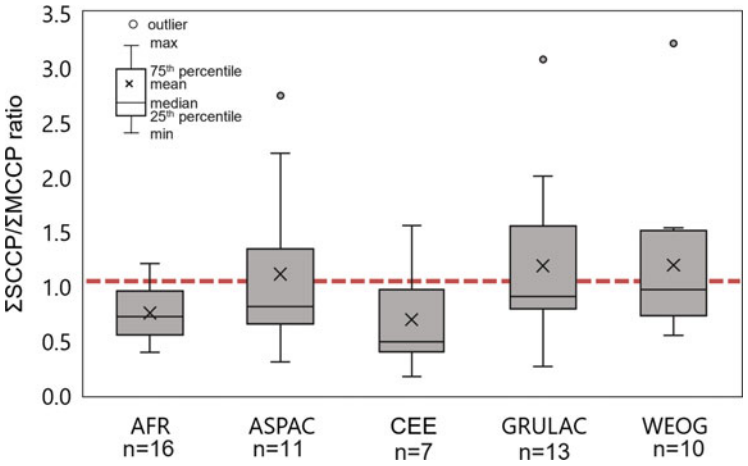

Fig. 8 Range of SCCP/MCCP ratios, sorted by geographical area or grouping. The dotted horizontal line indicates ratio = 1, i.e. equal presence of SCCP and MCCP. AFR, Africa; ASPAC, Asia-Pacific Group; CEE, Central and Eastern Europe; GRULAC, Group of Latin America and Caribbean Countries; WEOG, Western European and Others Group

53 countries where distinct data is available, MCCP even surpassed SCCP. Using the SCCP/MCCP ratio as an indication of this relation, it is interesting to see that African and Eastern European country samples had a tendency toward a higher dominance of MCCP, whereas in the other UN Regional Groups, median SCCP levels were closer to equal distribution compared to MCCP (AFR 58% MCCP, ASPAC 55%, GRULAC 53%, CEE 67%, WEOG 54%).

Interestingly, the pooled European samples seem to have a different SCCP/MCCP distribution in comparison with the samples of human milk reported for individual European countries: While one English and two Swedish studies on small regional pools or individual samples showed a slight to very strong dominance of SCCP over MCCP (in contrast to a slight dominance of MCCP in the Swedish sample of 2019), another study on individual samples from Germany in 2011 indicated the opposite (Thomas et al. 2006; Hilger et al. 2011; Darnerud et al. 2012; Zhou et al. 2020). Similar to the German pool sample of the present study (collected 2019, 65% of the determined CP were MCCP), the median of all individual samples from the 2011 study had 66% MCCP (Hilger et al. 2011). Further comparison or interpretation of these data is however not advisable, as individual samples from one small geographical area will have a different distribution as representatively sampled pools of the whole country and data on comparability of the applied quantification methods are not always available. Additionally, the 2011 dataset included a large number of left-censored data.

The remarkable difference between most WEOG and CEE samples might, like previously mentioned, be explained by differences in consumer behaviour, CP production industries, and availability of MCCP-containing products as result of

global trade, but lack of data on both aspects does not allow for any conclusions in this regard.

There is no recent literature data on CP in human milk available for Latin American and African countries. Studies describing levels and SCCP/MCCP distributions in Chinese samples indicate a much higher level of contamination especially in China and more dominantly consisting of SCCP (Xia et al. 2017a; b). Other studies on Chinese, Korean and Japanese regional pooled samples unfortunately only determined SCCP levels, so no further information on other CP groups could be obtained (Cao et al. 2017; Yang et al. 2018). It seems that China as one of the predominant CP producing countries has a different contamination pattern and level than other Asian countries which most likely are primarily exposed through product use. Unfortunately, neither China nor India, which also produces copious quantities of CP, did participate in the studies presented here.

3.3 Relation to Other POPs 2015–2019

Considering concentrations of a broad spectrum of 28 recommended chlorinated and brominated analytes in the pooled human milk samples (Malisch et al. 2023c), ranking SCCP and MCCP among these other Stockholm Convention POPs as listed until 2019 becomes feasible. For ease of comparison, the following listed 27 POPs included in this ranking were sorted into two groups:

- **Pesticides**—aldrin, chlordane, chlordecone, dichlorodiphenyltrichloroethane (DDT), dicofol; dieldrin, endosulfan, endrin, heptachlor, α-hexachlorocyclohexane (HCH), β-HCH; γ-HCH; mirex, pentachlorobenzene, pentachlorophenol (including pentachloroanisole) and toxaphene
- **(Other) Industrial chemicals and by-products**—hexabromobiphenyl (HBB), hexabromocyclododecane (HBCDD), hexachlorobenzene (HCB), hexachlorobutadiene; polybrominated diphenyl ethers (PBDE: tetra- and pentabromodiphenyl ether; hexa- and heptabromodiphenyl ether; decabromobiphenylether), polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polychlorinated naphthalenes (PCN).

Apart from WEOG and CEE, SCCP and MCCP dominated the share of POPs grouped as industrial chemicals and by-products in most areas (Fig. 9). Especially in continental European countries, the well-documented legacy POP problem with PCB (Breivik et al. 2007) apparently led to a higher share of industrial chemicals in human milk samples (Costopoulou et al. 2006; Abballe et al. 2008; Fång et al. 2015; Antignac et al. 2016; Malisch et al. 2023d, e). However, no further conclusions as to the source(s) or time frame of the PCB exposure are possible, since the investigation of POPs sources was not the aim of the studies presented here. A more detailed description of POPs distribution and likely sources can be found in part one of this book (Fürst 2023) and, with more detail on regional distribution and

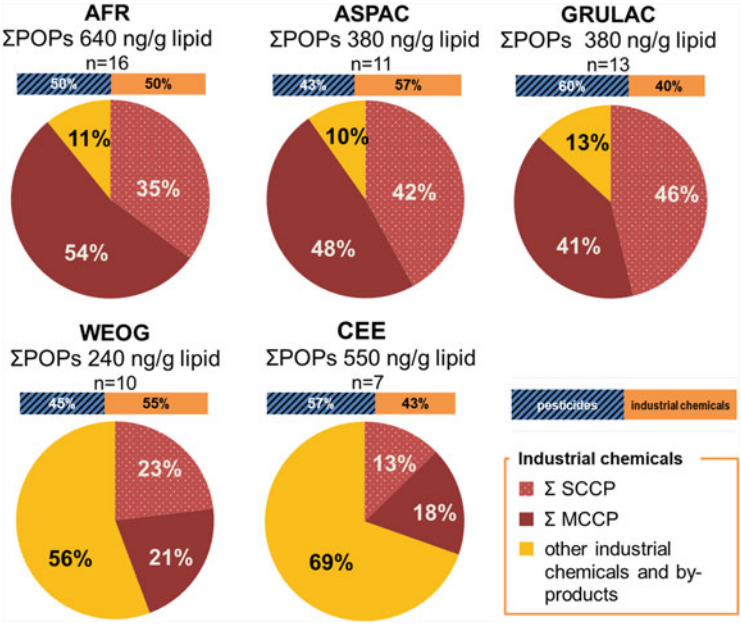

Fig. 9 Median sum of all POPs except PFAS analysed in pooled human milk samples from 2015–2019, sorted by UN regions and broken down into Stockholm Convention POPs groups (bar charts) and further into components of the 'industrial chemicals' POPs group (pie charts). AFR, Africa; ASPAC,Asia-Pacific Group; CEE, Central and Eastern Europe; GRULAC, Group of Latin American and Caribbean Countries; WEOG, Western European and Others Group

trends, in the Stockholm Convention regional monitoring reports (Stockholm Convention 2022).

Compared to all other POPs analysed in the samples and listed above, the concentrations of the sum of SCCP and MCCP were in most cases only surpassed by the pesticide DDT and its metabolites, excepting only continental European countries (part of WEOG and CEE) with high shares of PCB (Krätschmer et al. 2021a). Notably, DDT and its metabolites dominated the pesticide group throughout all regions with share of more than 90% (Krätschmer et al. 2021a; Malisch et al. 2023a, b).

Similarly elevated levels of DDT were also found in other human milk studies in Italy (Abballe et al. 2008), Jordan (Al Antary et al. 2017), France (Antignac et al. 2016), India (Bawa et al. 2018), Taiwan (Chen et al. 2018), Tanzania (Müller et al. 2017) and Siberia (Mamontova et al. 2017). The most likely reason for high DDT exposure in warmer climates is the use as insecticide to combat malaria (Conference of the Parties of the Stockholm Convention 2013). Additionally, a 2001 study with women of the Mohawk nation was also able to link higher levels of DDT and HCB in human milk to increased fish consumption of some of the participants (Fitzgerald et al. 2001), so it is likely that several factors have influenced DDT exposure (e.g.,

food chain, aim and time of restriction of use in countries/UN regions), similar as illustrated for main exposure pathways to CP in a previous section of this article.

As fish only plays a very minor role in dietary intake in most European countries (EFSA 2011), the elevated levels of CP and other POPs found in fish on the European market (Parera 2013; Fernandes et al. 2018; Krätschmer et al. 2019, 2021b; Labadie et al. 2019) are expected to only have a minor influence on CP level in human milk from that area (Albers et al. 1996).

4 Considerations on CP Homologue Patterns

4.1 UN Regional Group Characteristics

While concentrations of SCCP and MCCP are important from a risk assessment point of view, the homologue group patterns of samples can give indication on possible contamination sources or at least the severity of contamination with certain chlorination degrees or CP chain lengths. Homologue group patterns have previously been shown to be specific to geographical regions or species (Zhou et al. 2018; Krätschmer et al. 2019). When comparing these homologue group patterns, two aspects should be regarded:

(a) Distribution of alkyl chain lengths (Fig. 10) and
(b) Distribution of numbers of chlorine atoms (Fig. 11)

The median distribution of alkyl chain lengths in the five UN Regional Groups shows that SCCP (C_{10}–C_{13}) are still very present in all parts of the world, in

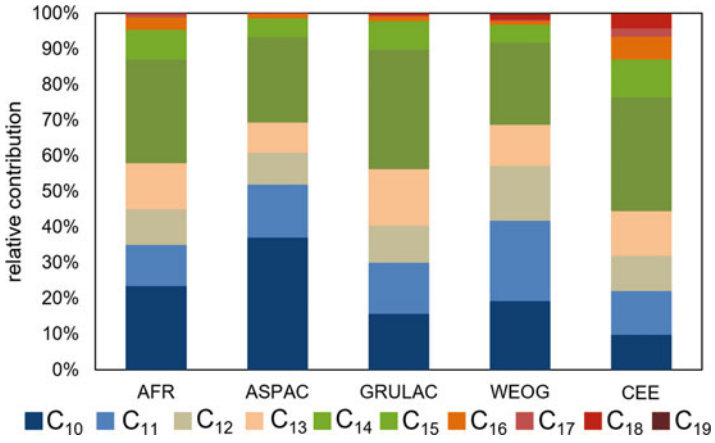

Fig. 10 Relative contribution of alkyl chain lengths C_{10}–C_{19} to the median CP homologue patterns for each UN Regional Group. Data derived using GC-ECNI-HRMS. AFR, Africa; ASPAC, Asia-Pacific Countries; CEE, Central and Eastern Europe; GRULAC, Group of Latin American and Caribbean Countries; WEOG, Western European and Others Group

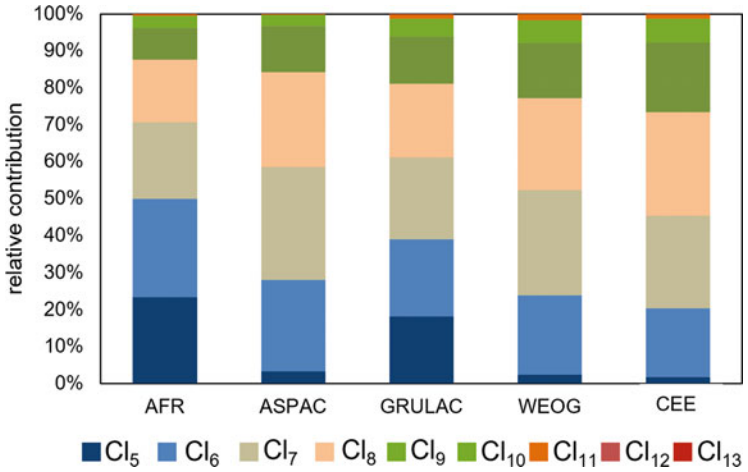

Fig. 11 Relative contribution of different chlorine atom numbers to median homologue patterns of the five UN Regional Groups. Data derived by GC-ECNI-HRMS with application of ECNI correction factors (Mézière et al. 2020). AFR, Africa; ASPAC, Asia-Pacific Countries; CEE, Central and Eastern Europe; GRULAC, Group of Latin American and Caribbean Countries; WEOG, Western European and Others Group

agreement with the previously discussed SCCP/MCCP ratios. Interestingly, the amount of chlorinated decanes (C_{10}-CPs) seems to have the highest variation among SCCP, with especially high shares in ASPAC sample pools. Such a distinct change in overall CP pattern might indicate a contamination source possibly typical for this area especially for the Pacific islands as they present most countries included in this group. Another interesting observation is the dominance of chlorinated tetradecanes (C_{14}-CPs) among MCCP (C_{14}–C_{17}), but also among the whole CP pattern. This is in accordance with findings in environmental samples (Krätschmer et al. 2019). Even though they were not quantified during this study, some LCCP (C_{18}–C_{20}), here especially chlorinated octadecanes (C_{18}-CPs), were detected in 8 European and 14 African sample pools.

Given the increasing reports of LCCP (C_{18}–C_{20}) and vLCCP (C_{21}–C_{36}) in food (e.g., Ding et al. 2021) and the environment (e.g., Yuan et al. 2021), a further increase of these CP groups can also be expected in human milk studies in the future. With CP being used in cable insulations and other parts of electronic equipment (Wang et al. 2018), mismanaged consumer product and electronic waste is a potential contamination source that is likely to show up in human surveys in addition to the primary use of the products themselves (Perkins et al. 2014). This might especially be of importance for countries with considerable amounts of e-waste recycling or large dumping sites outside government control, like it was reported in the past for Ghana and Nigeria (Schmidt 2006; UNEP 2018). Currently, e-waste management is regulated in eight African countries including Egypt, Ghana, Nigeria and Côte d'Ivoire, who are part of this human milk survey (Forti et al. 2020). In East Africa, a group of countries including the study participants Tanzania,

Uganda and Kenya have published a common strategy paper on e-waste management 2017, though progress in implementation varies (EACO 2017). While this possible exposure pathway seems valid for a variety of compounds, present data on pooled human milk samples from the first (2008–2010) and second sampling periods (2015–2019) do not show a uniform increase or decrease of CP levels in the mentioned countries, likely surpassed by other predictors and natural variations between study subjects.

In general, discussions of homologue group patterns always necessitate knowledge of the applied instrumentation and correction measures to evaluate and compare results (Krätschmer and Schächtele 2019; EURL POPs 2021). As is clearly shown in Fig. 11, chlorine numbers above 10 play a negligible role, whereas the sum of six to nine chlorine atoms describes 43–82% of the detected CP homologue groups. McGrath et al. proposed a method only evaluating homologue groups with six and seven chlorine atoms and validated this for different food matrices (McGrath et al. 2020).

However, application of this method on the human milk samples in the present studies would lead to a significant underestimation of CP concentrations: Only the African pooled samples would be described to a degree of more than 50%, whereas all other median area patterns contain homologue groups with six and seven chlorine atoms only to 22–30%. It is therefore uncertain if the screening method of McGrath et al. (2020) would still adequately quantify the overall amount of CP present in those sample pools. ASPAC, WEOG and CEE samples seem to be similar in their overall chlorination degree with low amounts of five chlorine atoms and the increasing presence of decachlorinated alkyl chains.

Interestingly, similarities between African and European sample pools apparent in the comparison of alkyl chain length patterns are not reflected in the chlorination levels (Fig. 11): the median Cl pattern of African pool samples features a much larger share of pentachlorinated CP (>20%) than the European sample pools (<3).

The difference in number of chlorine atoms has different impact on SCCP, MCCP and LCCP due to their increasing carbon chain lengths (Fernandes et al. 2022). For example, the mean number of chlorine atoms in C_{10}-CP in the WEOG area is 6.7 (Fig. 12a), translating into a mean chlorination degree of 63% (Fig. 12b). In comparison, the mean chlorine number for C_{14}-CP in Africa is also 6.7, but it translates into only 55% Cl for this CP chain length. However, some variation between individual samples is to be expected, as the patterns discussed here are median values of several pooled samples which themselves consist of numerous individual samples.

4.2 Sub-groups within UN Regional Groups

The UN Regional Groups used throughout this article are a political and economic construct, often formed in the early 1960s (UN General Assembly 2000). As such, they do not necessarily reflect similar population characteristics or environmental influences, which might lead to observable changes in CP levels. While the African

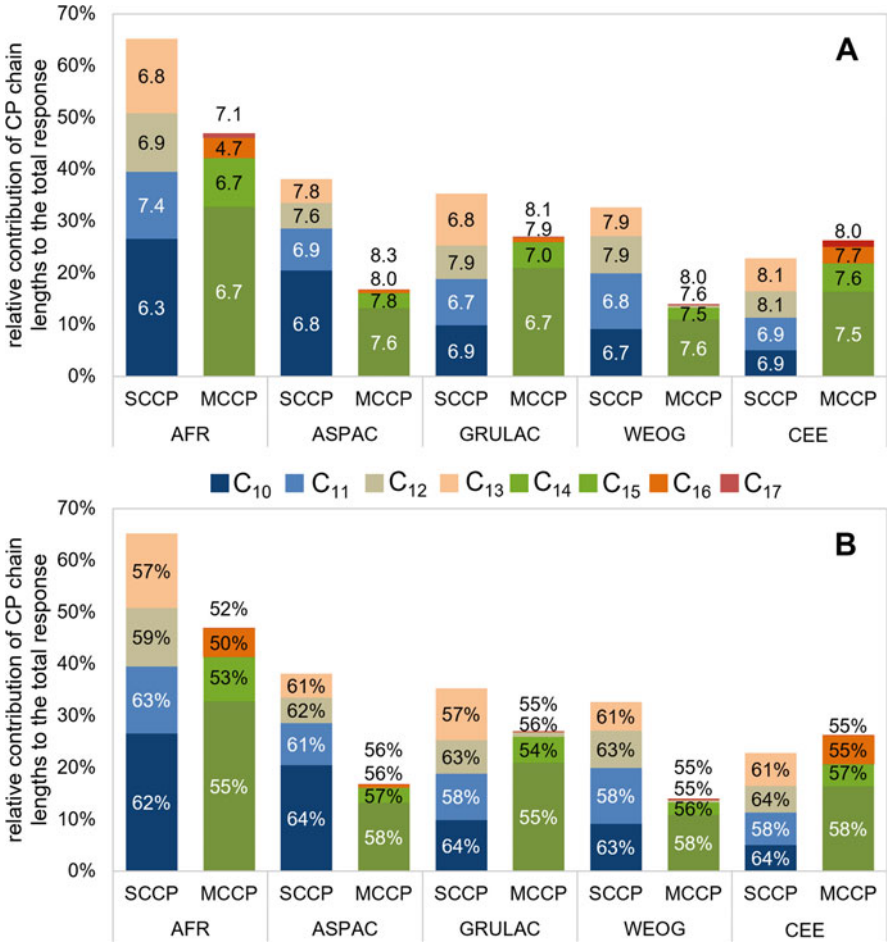

Fig. 12 Relative contribution of different SCCP and MCCP chain lengths to median homologue patterns of the five UN Regional Groups. Annotated are (**a**) the mean number of chlorine atoms and (**b**) mean relative chlorination degree per CP chain length for each area. Data derived by GC-ECNI-HRMS with application of ECNI correction factors (Mézière et al. 2020). AFR, Africa; ASPAC, Asia-Pacific Countries; CEE, Central and Eastern Europe; GRULAC, Group of Latin American and Caribbean Countries; WEOG, Western European and Others Group

group and the GRULAC countries are defined by geographical boundaries, especially WEOG countries and the ASPAC group combine countries of vastly different characteristics and locations. These differences have been previously addressed at 25th plenary meeting of the 55th United Nations General Assembly, where the formation of an Oceania regional group including Australia and New Zealand was proposed by the representative of Nauru (UN General Assembly 2000). In the interest of a closer evaluation of the results in the ASPAC region, three sub-groups were made for the countries participating in these studies: AP-1 (Pacific

Table 3 List of the countries in each sub-group of ASPAC

AP-1	AP-2	AP-3
Fiji	Cambodia	Mongolia
Kiribati	Indonesia	Tajikistan
Marshall Isl.	Thailand	
Palau	Vietnam	
Samoa		
Solomon Isl.		
Vanuatu		

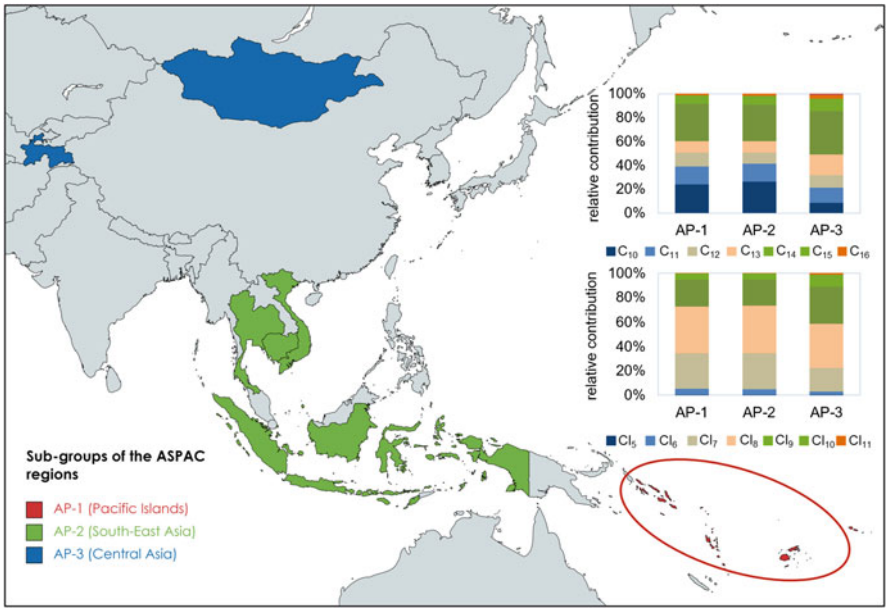

Fig. 13 Map of the sub-groups within the ASPAC region and relative contributions of carbon chains and chlorine numbers to median homologue group patterns for each of them. For conciseness, not all Pacific Island countries named in Table 3 are shown on this map

Islands), AP-2 (South-East Asia) and AP-3 (Central Asia). The exact grouping is shown in Table 3. Between these sub-groups, strong differences in SCCP and MCCP levels, average chlorination degrees and median homologue group patterns could be observed only for AP-3 (Fig. 12).

Between these sub-groups, strong differences in SCCP and MCCP levels, average chlorination degrees and median homologue group patterns could be observed only for the Central Asia sub-group (Fig. 13). Although the South-East Asian countries present more MCCP than the Pacific Island countries evaluated here, the rest of the overall patterns both in terms of chain length distribution and chlorine distribution are very similar. This might indicate different CP sources on the Pacific coast and further inside continental Asia. As only pattern data is available for

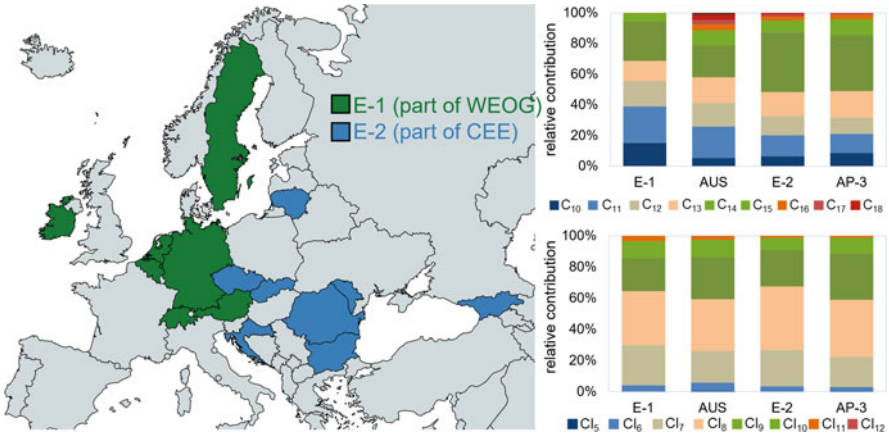

Fig. 14 Map of Europe with countries participating in this survey highlighted in green (E-1, belonging to the Western Europe and Others Group WEOG) and blue (E-2, belonging to the Central and Eastern European countries group CEE). Column diagrams on the right indicate the median carbon chain patterns and median chlorination patterns of E-1, E-2, Australia (AUS) and Mongolia (AP-3)

Mongolia in sub-group AP-3, no conclusions can be made on product streams or likely source countries.

Without the participation of the USA or Canada in the surveys presented here, the WEOG region consists of Western European countries and Australia. Geographically, some of the European WEOG countries are directly neighbouring countries of the CEE group (Fig. 14). A comparison of the median carbon chain and chlorine distribution patterns confirms the alleged differences between European WEOG countries and Australia, as the latter has visible contributions of longer MCCP and even C_{18}-LCCP in its median pattern. Interestingly, the median pattern of CEE countries does not match the other European countries, mostly due to a higher contribution of C_{14}-MCCP and octachlorinated homologue groups to the overall pattern.

However, this specific carbon chain pattern matches reasonably well the pattern of AP-3, the Central Asian sub-group including Mongolia already shown in Fig. 13. While the median chlorine distributions do not completely match, the pattern similarities are enough to suggest at least some overlap in CP sources between Central/Eastern Europe and Mongolia. Common economic pathways are likely to have played a part in the distribution of either CP mixtures or products containing CP in this area, at least in the past if not at present. Unfortunately, we do not have a Chinese pooled sample to compare patterns with, so it remains unclear if the present findings originate from the distribution of CP (formerly) produced in Eastern Europe (ECHA 2008; Euro Chlor 2021) or in China.

These two examples have shown that there are several different ways of grouping the countries participating in the UNEP human milk surveys. While the grouping by official UN Regional Groups as practised throughout this article is a valid option,

some connections—or differences—can only be shown when a more geographically-based approach to grouping is chosen. As a variety of economic, political, geographical and social factors are likely to play a role in the distribution of and consequently exposure to CP, any kind of group approach will always limit the insight into sources for individual countries. This article is merely meant to give a first insight into available data and encourage competent authorities to investigate further.

4.3 Differences Depending on Sampling Region

Sampling guidelines for the UNEP human milk surveys describe the pooled samples of each country to be representative of the background contamination of the country's citizens (UNEP 2017). However, even with careful screening and choosing of the participants, differences in consumer preferences, undisclosed or unknown contamination sources or even differences in food choices might influence the resulting CP homologue pattern of the pooled sample.

For example, in 2013, Brazil submitted three different samples, which varied considerably in the CP content, namely by a factor of ~3 for SCCP (17–60 ng/g lipid) and by a factor of ~8 for MCCP (5.5–40 ng/g lipid). These samples were collected in three different greater regions of Brazil: the barely populated North Region, additionally including the central-west federal states Mato Grosso and Mato Grosso do Sul (Northwest, BR-GR1); the Northeast Region (BR-GR2); and the densely populated South and Southeast Regions additionally including the Federal District and Goiás (BR-GR3). These differences in region and population are also visible in the homologue group patterns of these samples (Fig. 15).

Most strikingly, the homologue group pattern of the sample pool from the southern federal states including the Federal District (BR-GR3) showed a completely different CP pattern than the other two, emphasizing MCCP and LCCP with lower chlorination degrees much more than the other patterns. It is at the same time also the pool with the highest amount of SCCP and MCCP quantified among the three. While the BR-GR1 and BR-GR2 pools share some similarities on the first look, the sample pool from the Northeast Region (BR-GR2) has an overlying higher chlorinated SCCP pattern (C_{10}–C_{13}, Cl_9–Cl_{11}) similar to known technical SCCP mixtures (Schinkel et al. 2018; Sprengel and Vetter 2019; Fernandes et al. 2022, example in Fig. 16).

In this case, further follow-up investigations might be advisable, as such a Gaussian distribution over several alkyl chain lengths is highly unusual in a metabolized sample. Therefore, in different regions of a country, various patterns can be observed. Such findings underline the need for robust, representative sampling in all areas of a participating country for the pooled sample. A follow-up with analyses of individual samples could reveal the ranges of CP concentrations and variation of patterns within Brazil or any other country with unusual homologue pattern distributions in the pooled samples.

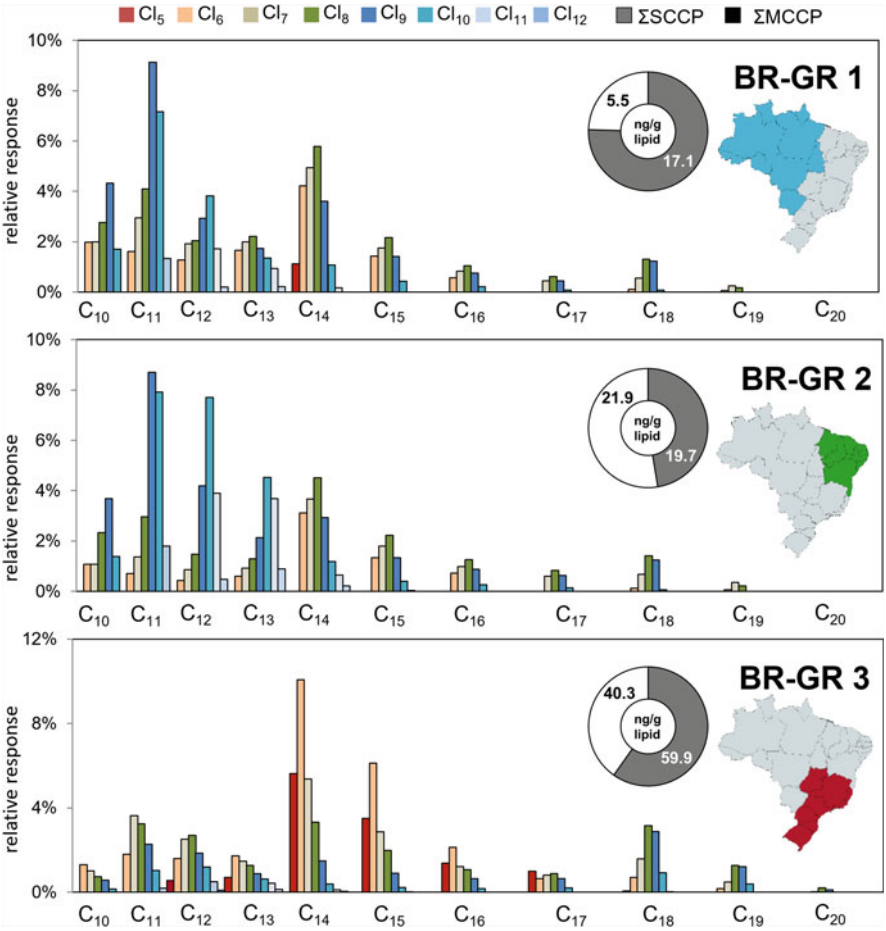

Fig. 15 CP homologue group patterns for human milk pooled samples collected in three greater regions of Brazil 2012 with indicated sum concentrations of SCCP and MCCP. Patterns derived from GC-ECNI-HRMS data with correction factors for lower chlorinated homologue groups. BR-GR1: North Region, with Mato Grosso and Mato Grosso do Sul; BR-GR2: Northeast Region; BR-GR3: South Region and Southeast Region, with additional states Federal District and Goiás

While the correlation between age of the mothers and level of different (non-CP) chlorinated contaminants in human milk could be proven as early as 1996 (Albers et al. 1996), a newer study on CP in human milk from 2021 revealed no correlation between CP levels and age, height, weight, fish consumption or place of residence (urban/rural) of the participating mothers (Krätschmer et al. 2021a). With next to no data available on metabolization of CP and correlated changes in homologue group patterns, this might be an avenue worth investigating in the future, also to better understand such regional differences as shown here for Brazil.

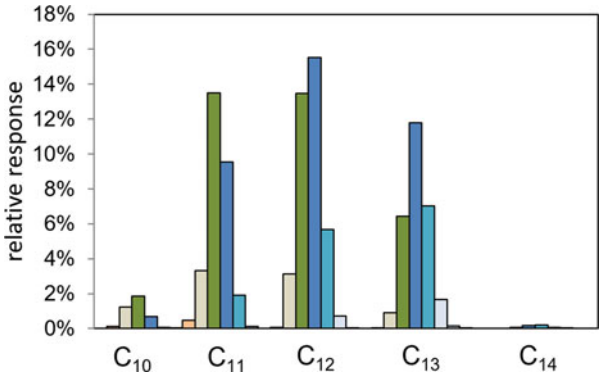

Fig. 16 Homologue group pattern of an SCCP mixed standard (Chloroparaffin C_{10}–C_{13} 63% Cl, Dr. Ehrenstorfer, Augsburg, Germany) exhibiting the typical Gaussian distribution of responses also found in technical mixtures

5 Conclusions

While not being the primary or only tissue to bioaccumulate lipophilic contaminants, the levels of POPs found in human milk of primiparous mothers still represent their accumulated exposure since birth, partly modified by metabolism and further losses through excretion (Albers et al. 1996). Therefore, comparing background contamination levels in human milk samples with data on environmental (Fridén et al. 2011; Hilger et al. 2013; Gao et al. 2016; Brits et al. 2019) or domestic (Schinkel et al. 2019; Wang et al. 2019) samples can help identifying additional contamination sources not indicated in dietary intake studies. While such information is important for better understanding CP contamination across the globe, especially for breastfed infants, the amounts of CP found in human milk as their primary source of sustenance are decisive for intake.

The human milk studies of the United Nations Environment Programme (UNEP) were not designed to point out specific contamination hotspots or to accurately determine exposure levels. On the contrary, the focus on pooled samples collected from participants that are expressly not living near known contamination hotspots is conducive to UNEPs goal of monitoring changes in the overall background contamination levels in humans as part of an effectiveness evaluation of POPs regulations.

In the WHO/UNEP sampling campaigns presented here, CP were found in all pooled samples from five UN Regional Groups with levels ranging from 9 to 700 ng/g lipid. Compared to other compounds classified as industrial chemicals by the Stockholm Convention on POPs, the sum of SCCP and MCCP dominated the findings in the pooled human milk samples from all UN regions. If the sample pools with human milk from donors without any known major contamination sources nearby already shown this consistent, and in some cases, high abundance of CP, individual samples from local population close to emission spots or as result of exposure to consumer products or in the domestic environment might be markedly higher.

The presence of MCCP, hereto mostly unregulated, could be established worldwide, often in equal or higher amounts compared to the now regulated SCCP. Indications of LCCP in samples from WEOG, CEE and GRULAC countries also give cause for concern and a need for further research in this area. Both findings show that merely regulating SCCP has led to a shift in production toward longer carbon chain lengths; a situation that should prompt evaluations of MCCP and LCCP and further regulatory efforts in this area. CP production already surpasses the total PCB production volume (1.0–1.5 million tons, Breivik et al. 2007) in a single year and the 20–25% of the PCB production volume that became 'environmentally available' still have a major impact in the human milk samples in some countries (Grimm et al. 2015; Malisch et al. 2023e). It is to be expected that the amount of CP released into the environment will continue to rise, likely surpassing also the cumulative amount of DDT (currently ~2.8 million tons, ATSDR 2022) soon. Consequently, levels of all types of CP can also be expected to keep rising and become a steady presence in human milk for decades to come.

While available data represent a pool of several individual samples, the determined average CP homologue group patterns of each country sample allow for regional grouping consistent with geographic or economic relations of the countries. Contrastingly, evaluation of CP homologue group patterns in samples collected in specific parts of the same country illustrate the large regional variations that disappear within the average country patterns. Efforts to lower CP exposure therefore need to evaluate sources at a more local level, taking into account regional economic and nutritional variations, consumer behaviour and possible re-use of legacy products. Nevertheless, all evaluated CP homologue group patterns presented some common markers, i.e. the leaning toward higher chlorinated homologues and a general dominance of chlorinated decanes, undecanes and tetradecanes (C_{10}-, C_{11}- and C_{14}-CPs) within most patterns, although the chlorinated decanes vary markedly in some of the pooled samples. Further research is needed to establish whether these commonalities stem from similarities in the technical CP products or from metabolization/distribution within the human body.

Acknowledgements This publication was developed in the framework of the projects titled 'Implementation of the POPs Monitoring Plan in the Asian Region' and 'Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region', funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

The authors would like to thank all the participating countries, the national and regional coordinators for their commitment and hard work in these human milk studies. The team of Chemical and Veterinary Institute (CVUA) Freiburg is gratefully acknowledged for analysing the samples discussed here in their role as UNEP Reference Laboratory for POPs in human milk. Data

analysis for determination of time trends was supported by the RECETOX Research Infrastructure (No. LM2018121) and the CETOCOEN EXCELLENCE Teaming Phase 2 project financed by the European Union's Horizon 2020 (No 857560) and the European Structural and Investment Funds (No. CZ.02.1.01/0.0/0.0/17_043/0009632). The two anonymous reviewers are also acknowledged for their valuable insights and commentary that allowed further improvement of this manuscript and allowed to develop additional perspectives for data evaluation.

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WHO- and UNEP-Coordinated Exposure Studies 2000–2019: Findings of Polychlorinated Naphthalenes

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Abstract

The concentrations of polychlorinated naphthalenes (PCN) were determined in 40 pooled human milk samples from 39 countries covering all five of the United Nations regional groups. The samples were collected in the 2016–2019 exposure studies on persistent organic pollutants coordinated by the United Nations Environment Programme (UNEP).

The median concentration of the sum of 26 PCN was 55 pg/g lipid (range 27 pg/g to 170 pg/g). Human milk from European countries showed considerably higher levels than those found in milk from countries in the African, Asia-Pacific, and Latin America/Caribbean regions. The most abundant congeners were the congener pairs PCN 52/60 and PCN 66/67 (inseparable by conventional chromatography) and to a lesser extent PCN 28/36, PCN 42, PCN 46, PCN 48, PCN 59, and PCN 69.

Among other adverse biological effects, a critical response of many PCN congeners is dioxin-like toxicity. So, in addition to reporting concentrations of

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_11

individual congeners, the toxic equivalents (TEQ) were also calculated in these samples, using two sets of relative effect potency (REP) values: a set that has been used in a number of human exposure studies and another set reported by Falandysz et al. (J Environ Sci Health, Part C: Environ Carcinogenesis Ecotoxicol Rev 32(3):239–272, 2014). The median PCN-TEQ concentration in human milk was 0.07 pg PCN-TEQ/g lipid (range 0.03 pg/g to 0.23 pg/g), when calculated using the human biomonitoring study REPs, and 0.03 pg PCN-TEQ/g lipid (range 0.01 pg/g to 0.10 pg/g), when calculated with other suggested REPs. The vast majority, about 90%, of this TEQ can be attributed to the PCN 66/67 congener pair. Individual REPs for PCN 66 and 67 from in vivo studies are quite different, but a chromatographic separation of these two congeners is not possible under routine GC conditions. Different approaches to estimate the uncertainties showed that the value of the REPs used is more important than the analytical problem to separate PCN 66 and PCN 67. PCN-TEQ based on the two sets of REPs differ approximately by a factor of 2.2, whereas the congener-specific determination was estimated to result in approximately 30% lower concentrations in comparison with the standard method.

The assessment of PCN 66 and PCN 67 in order to obtain confirmed TEF would be most important for calculations of the dioxin-like toxicity of PCN, followed by PCN 69. Minor contributions to PCN-TEQ concentrations in human milk come from PCN 52/60, PCN 64/68, PCN 70, and PCN 73.

On average, the contribution of PCN-TEQ to the cumulative TEQ (including the overall sum of toxic equivalents of PCDD, PCDF, and dioxin-like PCB [WHO_{2005}-TEQ]) is between 1% and 2%, with a wider range of up to 5% for the 39 countries of this study. This is about an order of magnitude lower than the contribution of dioxin-like PCB to the cumulative TEQ (median 26%). In line with the observed higher total PCN concentrations, European countries also showed considerably higher levels of PCN-TEQ than found in the other regions. PCN-TEQ calculated with REPs used in human biomonitoring studies add on average about 2% to the cumulative TEQ of dioxin-like contaminants in Africa, the Asia-Pacific region, and Latin American and Caribbean countries and about 4% in European countries. The corresponding contribution of PCN-TEQ calculated using the other set would be 1% in non-European countries and 2% in European countries.

Keywords

Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · Polychlorinated naphthalenes (PCN) · Dioxin-like toxicity · Toxic equivalents (TEQ) · Global WHO/UNEP human milk studies

1 Introduction

Polychlorinated naphthalenes (PCN) were produced during much of the last century, principally from the 1930s to the 1980s. They were used in multiple applications such as adhesives, dielectrics, flame (fire) retardant, fuel additives, fungicides, impregnating agents, insecticides, lubricants, plasticizers, solvents, stabilizers, wood preservatives, etc. The combined total global production volumes of technical formulations were estimated to range from 150,000 to 400,000 tons (Falandysz 1998; Falandysz and Fernandes 2020). In addition to this industrial production, PCN are also formed and released unintentionally during combustion processes such as the incineration of waste, and during metal and cement production as well as during fires of any kind including large-scale bush and forest fires. The magnitude of such sources and implication for human exposure is largely unknown. They were also formed as by-products during the manufacture of other large-volume industrial chemicals such as polychlorinated biphenyls (PCB) (Taniyasu et al. 2003, 2005; Yamashita et al. 2000). The production, properties, applications, environmental distribution, contamination of food, occurrence in human tissues and other relevant background information on PCN have been extensively described in earlier reviews (Jakobsson and Asplund 2000; Falandysz 2003; Fernandes et al. 2017).

As chemically and physiologically stable substances, PCN are ubiquitous and persist in environmental and biological systems, including humans. They have been detected in significant quantities in sediments, biota and water and even from remote regions such as the Arctic (Bidleman et al. 2010). These compounds are also lipophilic, which results in their biomagnification in the food chain and bioaccumulation in fatty tissues of animals and humans. These properties together with concerns about their toxicity have resulted in them being classified as persistent organic pollutants (POPs). In order to protect human health and the environment, the Stockholm Convention on POPs has listed certain chemicals with the intention of eliminating production or restricting their release into the environment (UNEP 2001). In 2015, PCN were listed to Annexes A (Elimination) and C (Unintentional release) under this Convention (UNEP 2020). A draft guidance on preparing PCN inventories provides information on production and possible sources. Furthermore, it enables the collection of information for the development of effective strategies for the elimination of PCN and the environmentally sound management of products, stockpiles, and wastes containing PCN (UNEP 2017). All listed POPs, including PCN are subject to Article 16 of this Convention, which requires that they should be monitored in order to evaluate the effectiveness of the remedial actions applied. The analysis of these POPs in human milk as one of the core monitoring matrices has been recommended within the framework of the Global Monitoring Plan on POPs (GMP) (UNEP 2019).

Exposure to PCN reportedly provokes a number of toxic responses ranging from hepatotoxicity, neurotoxicity, and immune response suppression along with endocrine disruption (Fernandes et al. 2022a). Historically, these effects were seen in workers occupationally exposed to high level levels of PCN (Hayward 1998) which is now unlikely following the end of widescale PCN production and use. Some of

these effects have been studied more recently in test animals (Kilanowicz et al. 2015; Klimczak et al. 2018; Stragierowicz et al. 2015, 2018) at doses of sub- to low-mg kg^{-1} of bodyweight. In current times, human exposure to PCN is likely to be lower and arise through non-occupational pathways (environmental and dietary intake) with more subtle and sensitive endpoints (Fernandes et al. 2022a). Exposure to some PCN congeners results in toxicological responses that are similar to 2,3,7,8-TCDD (dioxin-like toxicity) (Engwall et al. 1994; Villeneuve et al. 2000; Suzuki et al. 2020; Fernandes et al. 2022a). The most studied of these PCN congeners have the ability to bind with varying degrees of potency to the aryl hydrocarbon receptor (AhR) in line with their PCDD/PCDF analogs, the most potent of which include congeners such as 2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD, etc. The preliminary stage of the biochemical mechanism that initiates many of the potent long-term toxic effects of these compounds on vertebrate species is through activation of the AhR (Denison et al. 2002; Falandysz et al. 2014; Fernandes et al. 2022b). Therefore, the inclusion of PCN in the Toxicity Equivalency Factor (TEF) concept for dioxin-like compounds has been suggested (Van den Berg et al. 2006).

The World Health Organization (WHO)- and United Nations Environment Programme (UNEP)-coordinated exposure studies on POPs in human milk are reviewed in the different parts of this compendium covering a variety of aspects of analysis, exposure, and potential adverse health effects for the breastfed infant. The general introduction (Part I) gives an overview of the WHO/UNEP-coordinated exposure studies performed between 1987 and 2019 (Malisch et al. 2023a), a review of human milk surveys on POPs (Fürst 2023) and a review of the Stockholm Convention on POPs and its implementation summarized by regional and global monitoring reports (Šebková 2023). Analytical aspects, including methods used for determination of PCN in samples of the WHO/UNEP-coordinated exposure studies and their validation (Schächtele et al. 2023), are presented in Part II. In various articles in Part III, the findings for chlorinated and brominated POPs between 2000 and 2019 are reported and discussed, including here for PCN. Part IV presents an assessment of time trends and a risk–benefit analysis for the breastfed infant deriving from dioxin-like compounds and Part V provides a summary of all findings and conclusions.

All substance-specific data of the WHO/UNEP-coordinated exposure studies are deposited at the Global Monitoring Plan Data Warehouse (GMP DWH), which can be publicly accessed. This serves as the source of information for the regional and global reports of the GMP and for the evaluation of the effectiveness of the Convention to eliminate or reduce emissions of selected POPs (Global Monitoring Plan Data Warehouse 2020).

In this article, the PCN results for 40 pooled samples from 39 countries collected between 2016 and 2019 are discussed.

2 General Aspects

2.1 Link to the General Introduction (Countries, UN Regions, Protocol, Samples)

An overview of the scope, protocols for collection of samples, expansion of analytes of interest over time in terms of inclusion in the Stockholm Convention, participation of countries with classification in UN regional groups and temporal differentiation are given in the general introduction in Part I (Malisch et al. 2023a). In brief, the collection of a number of individual samples and preparation of representative pooled samples in all rounds were supervised by a national coordinator in each country following the WHO/UNEP-standardized protocols. Equal aliquots of the individual samples collected within a country were combined to produce a composite, which was considered representative to provide data on the average levels of POPs for that country or a subpopulation at the time of sampling. The pooled samples were then sent to the WHO/UNEP Reference Laboratories for analysis.

40 pooled samples from 39 countries collected between 2016 and 2019 were analyzed for PCN. The detailed data for all samples is contained at the POPs GMP Data Warehouse and can be publicly retrieved (Global Monitoring Plan Data Warehouse 2020).

In accordance with the implementation of the GMP, parties report flexibly through one of the five United Nations regional organization groups. Therefore, countries are classified according to one of these five UN geopolitical groups: (1) African Group, (2) Asia-Pacific Group, (3) Eastern European Group, (4) Group of Latin American and Caribbean Countries (GRULAC), and (5) Western European and Others Group (WEOG). For participating countries and regional distribution, see Malisch et al. 2023a. It should be noted that these quantitative results are not intended to be used for the ranking of individual countries.

2.2 Analysis

There are theoretically 75 PCN congeners (mono-chlorinated to octa-chlorinated) and in practice all occur at varying concentrations in technical products or are formed during thermal reactions (Hanari et al. 2013, 2015; Horii et al. 2004; Ieda et al. 2011). The GMP guidance (UNEP 2019) does not specify any specific congeners for analysis yet *("congeners to be decided").* For the UNEP-coordinated human milk survey 2016–2019, a set of 26 PCN congeners (Table 1) were used for establishment and performance indication of the analytical method that was developed and used. These congeners were chosen based on the toxicological characteristics, reported levels of occurrence, congener patterns, and the availability of analytical standards at times of method development and validation.

Table 1 PCN congeners covered by the applied analytical method

Congener number	Chemical structure
PCN 27	1,2,3,4-TetraCN
PCN 28/36 *	1,2,3,5-TetraCN/1,2,5,6-TetraCN
PCN 31	1,2,3,8-TetraCN
PCN 42	1,3,5,7-TetraCN
PCN 46	1,4,5,8-TetraCN
PCN 48	**2,3,6,7-TetraCN**
PCN 49	1,2,3,4,5-PentaCN
PCN 50	1,2,3,4,6-PentaCN
PCN 52/60 *	1,2,3,5,7-PentaCN/1,2,4,6,7-PentaCN
PCN 53	1,2,3,5,8-PentaCN
PCN 59	1,2,4,5,8-PentaCN
PCN 63	1,2,3,4,5,6-HexaCN
PCN 64/68 *	1,2,3,4,5,7-HexaCN/1,2,3,5,6,8-HexaCN
PCN 65	1,2,3,4,5,8-HexaCN
PCN 66/67 *	**1,2,3,4,6,7-HexaCN/1,2,3,5,6,7-HexaCN**
PCN 69	1,2,3,5,7,8-HexaCN
PCN 70	**1,2,3,6,7,8-HexaCN**
PCN 71/72 *	1,2,4,5,6,8-HexaCN/1,2,4,5,7,8-HexaCN
PCN 73	**1,2,3,4,5,6,7-HeptaCN**
PCN 74	1,2,3,4,5,6,8-HeptaCN
PCN 75	OctaCN

Congeners that based on their chlorine substitution pattern are expected to produce dioxin-like effects are indicated bold; congener pairs that were not chromatographically separated during measurement are marked as *

A full description of the internally standardized method, which uses eight ^{13}Carbon labeled PCN of the homologue groups TetraCN to OctaCN, and the results of the method validation have been presented earlier in Part II (Schächtele et al. 2023). In brief, samples were centrifuged and the upper cream layer was removed. After addition of sodium sulfate, the cream was extracted using hexane and purified on a multi-adsorbent column system on a DEXTechPlus sample preparation system using a standard silica column impregnated with sulfuric acid for fat degradation and thus for lipid separation. Purification and fractionation of the PCN from other contaminants such as PCB were achieved using alumina and activated carbon columns. The PCN congeners were measured on a high-resolution gas chromatograph coupled to a high-resolution mass spectrometer (HRGC/HRMS at a resolution of 10,000 at 5% peak height; Thermofisher DFS) using a DB-5 MS (60 m) column to separate the PCN congeners. Analytical confirmation measurement was carried out using GC-Orbitrap Q Exactive at a resolution of 60,000. All concentrations are reported on a lipid basis.

3 Results and Discussion

3.1 Sum of Measured PCN Congeners (∑ 26 PCN)

The median concentration of ∑26 PCN in 40 pooled human milk samples from 39 countries was 55 pg/g lipid (range 27 pg/g to 170 pg/g). The concentrations in samples from European countries were considerably higher than those found in the other regions included in this study (Africa, Asia-Pacific, and Latin America and Caribbean). The median concentration for Europe, at 152 pg/g, was three-fold higher than found in the other regions (Table 2). This higher PCN contamination in European human milk is reflected in the minimum concentration which at 86 pg/g is very close to the mean maximum level (93 pg/g) for the other three regions. This finding is not unexpected as the European region saw high levels of manufacture and use of PCN during the last century, as was reported earlier for North America (Hayward 1998; Falandysz et al. 2008; Noma et al. 2004). In the non-European countries included in this study (which do not include the North American region), the concentrations of ∑26 PCN were relatively low, ranging from 27 pg/g to 66 pg/g, apart from the Solomon Islands and Jamaica. Figures 1, 2, 3 and 4 illustrate the findings in countries of these regions.

3.2 PCN Patterns

Concurring with observations reported for human tissue samples as summarized in a review article (Fernandes et al. 2017), the most abundant congeners that were observed in the human milk samples were two penta-chlorinated and two hexa-chlorinated congeners, namely PCN 52/60 and PCN 66/67, and to a lesser extent, tetra-chlorinated congeners (PCN 28/36, PCN 42, PCN 46, and PCN 48), penta-chlorinated PCN 59 and hexa-chlorinated PCN 69. In Africa, the Pacific Islands and European countries, the abundance of PCN 52/60 is higher than that of PCN 66/67, which is in contrast to the Asian and Latin American countries that were included. Furthermore, in some countries of the Pacific Islands and Latin America and the Caribbean higher proportions of PCN 28/36 were found than in other countries/regions (Table 3; Figs. 5, 6, 7, 8, and 9). It is important to note that the patterns are at best an indication of the congener distribution in human milk for the different studied regions and may not be representative, as some countries where PCN were

Table 2 Range of concentration of the sum of 26 PCN congeners in the African region, the Asia-Pacific region, the Latin American and Caribbean region, and European countries

	N	Median	Min	Max
Africa	14	51.3	30.2	66.4
Asia-Pacific region	10	50.4	27.3	114
Latin America and the Caribbean	9	41.0	27.3	98.2
Europe	7	152	85.5	170

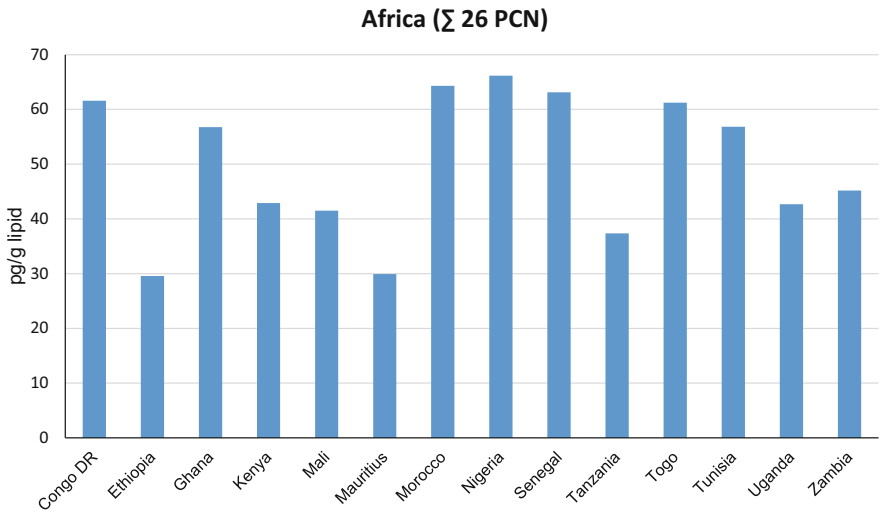

Fig. 1 Results of the 2016–2019 survey for PCN concentrations (sum of 26 congeners) in human milk in countries from Africa (pg ∑ 26 PCN/g lipid)

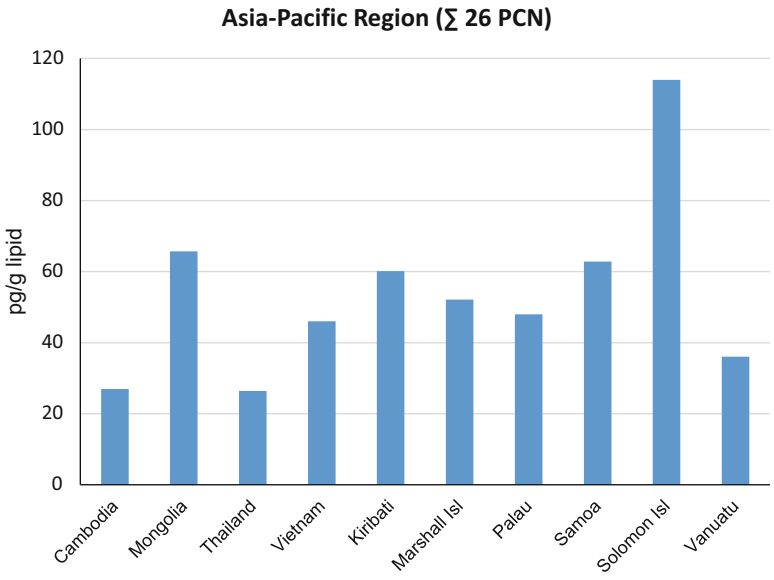

Fig. 2 Results of the 2016–2019 survey for PCN concentrations (sum of 26 congeners) in human milk in countries from the Asia-Pacific Region (pg ∑ 26 PCN/g lipid)

manufactured, e.g., UK, France, Italy, etc. (Hayward 1998; Falandysz et al. 2008; Noma et al. 2004) and may have seen higher levels of usage due to earlier industrialization, were not participants in this study.

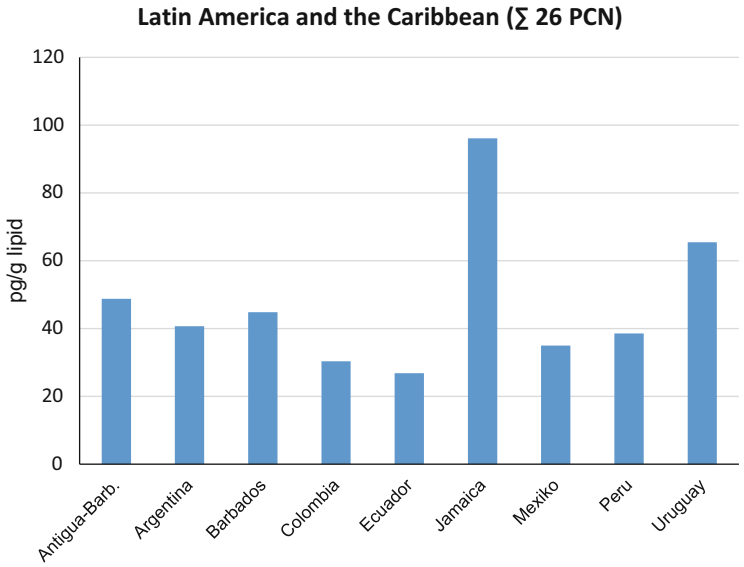

Fig. 3 Results of the 2016–2019 survey for PCN concentrations (sum of 26 congeners) in human milk in countries from Latin America and the Caribbean (pg ∑ 26 PCN/g lipid)

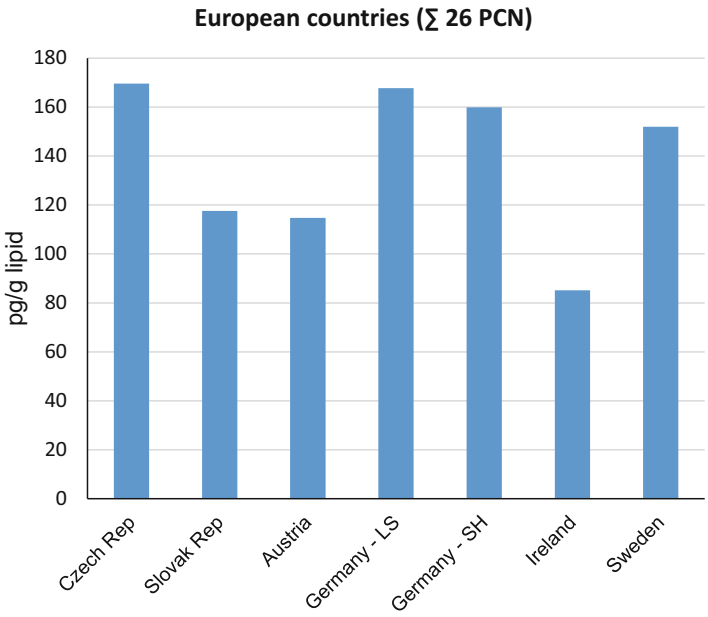

Fig. 4 Results of the 2016–2019 survey for PCN concentrations (sum of 26 congeners) in human milk in European countries (pg ∑ 26 PCN/g lipid)

Table 3 Pattern of PCN congeners (as % of ∑ 26 PCN) in countries from Africa, Asia, the Pacific Islands, Latin America, and the Caribbean and Europe. Congeners with higher REPs that make a greater contribution to TEQ are indicated in bold

	Africa			Asia			Pacific Islands			Latin America and Caribbean			European Countries		
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
PCN 27	0.2%	0.1%	0.6%	0.2%	0.1%	0.4%	0.2%	0.1%	0.3%	0.2%	0.1%	0.6%	0.1%	0.1%	0.2%
PCN 28/36	6.4%	2.6%	19.4%	9.8%	4.3%	13.8%	13.8%	2.5%	26.7%	11.7%	3.6%	45.2%	3.2%	2.3%	7.8%
PCN 31	0.2%	0.1%	0.4%	0.2%	0.1%	0.6%	0.2%	0.1%	0.3%	0.3%	0.1%	0.5%	0.1%	0.0%	0.1%
PCN 42	5.8%	3.2%	8.3%	5.4%	2.7%	12.5%	4.3%	2.6%	5.1%	3.4%	2.7%	5.3%	10.7%	7.6%	12.6%
PCN 46	2.7%	1.5%	6.1%	1.3%	0.7%	7.3%	1.8%	1.5%	3.1%	1.8%	1.5%	2.7%	4.2%	2.0%	5.7%
PCN 48	2.4%	1.8%	3.8%	2.4%	1.2%	3.6%	1.8%	1.2%	3.2%	2.4%	1.3%	4.2%	0.9%	0.5%	1.6%
PCN 49	0.2%	0.1%	0.4%	0.1%	0.1%	0.3%	0.2%	0.1%	0.2%	0.2%	0.1%	0.3%	0.1%	0.0%	0.1%
PCN 50	0.4%	0.2%	0.7%	0.4%	0.3%	0.5%	0.5%	0.3%	1.0%	0.4%	0.2%	0.6%	0.2%	0.1%	0.3%
PCN 52/60	36.0%	31.5%	44.7%	27.6%	20.6%	33.3%	35.4%	28.9%	43.2%	31.6%	21.4%	38.2%	39.9%	34.9%	48.6%
PCN 53	1.3%	0.3%	3.3%	0.3%	0.2%	1.0%	1.4%	0.7%	4.6%	0.8%	0.6%	1.7%	0.8%	0.5%	1.6%
PCN 59	3.8%	0.8%	7.7%	1.1%	0.9%	2.2%	4.0%	1.9%	8.7%	1.8%	1.1%	4.0%	1.9%	1.2%	3.0%
PCN 63	1.1%	0.2%	2.5%	0.6%	0.2%	0.9%	1.2%	0.5%	5.0%	0.6%	0.4%	1.3%	0.2%	0.2%	1.1%
PCN 64/68	0.6%	0.1%	1.0%	0.3%	0.1%	0.6%	0.6%	0.3%	2.1%	0.2%	0.2%	0.7%	0.2%	0.1%	0.5%
PCN 65	0.4%	0.1%	1.3%	0.2%	0.1%	0.5%	0.6%	0.2%	3.4%	0.2%	0.1%	0.3%	0.1%	0.1%	0.4%
PCN 66/67	27.1%	15.5%	34.0%	41.4%	37.8%	45.4%	19.2%	10.5%	27.1%	37.5%	17.9%	44.5%	28.9%	23.0%	39.5%
PCN 69	7.3%	4.0%	9.8%	3.6%	1.5%	6.7%	7.0%	3.0%	16.2%	4.1%	2.6%	7.1%	4.0%	2.1%	6.6%
PCN 70	0.2%	0.1%	0.3%	0.5%	0.1%	0.5%	0.1%	0.1%	0.3%	0.2%	0.1%	0.4%	0.1%	0.0%	0.1%
PCN 71/72	1.1%	0.1%	2.5%	0.3%	0.2%	0.4%	1.2%	0.4%	5.1%	0.4%	0.2%	1.9%	0.3%	0.1%	0.7%
PCN 73	0.9%	0.6%	2.2%	1.4%	0.8%	1.9%	0.8%	0.7%	1.1%	0.7%	0.5%	1.3%	0.5%	0.3%	0.7%
PCN 74	0.1%	0.0%	0.2%	0.2%	0.1%	0.3%	0.1%	0.1%	0.1%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%
PCN 75	0.1%	0.1%	0.4%	0.2%	0.1%	0.6%	0.1%	0.1%	0.3%	0.2%	0.1%	0.4%	0.1%	0.0%	0.1%

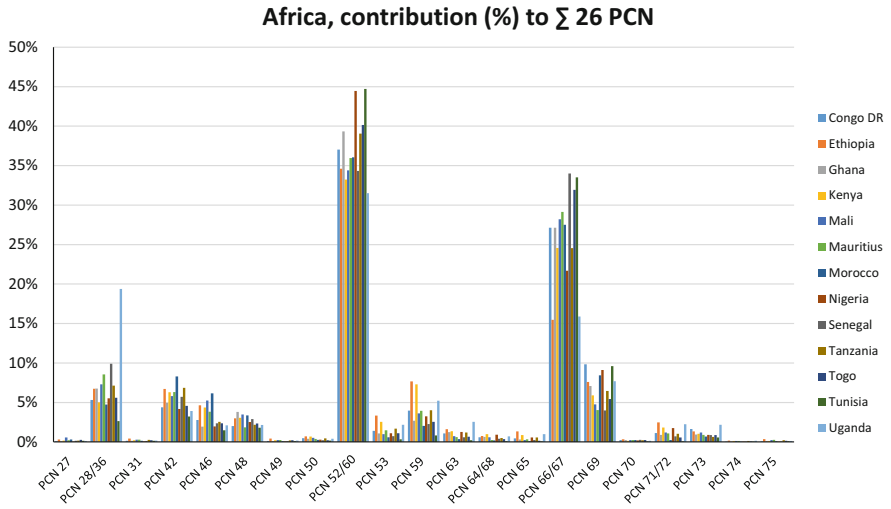

Fig. 5 Pattern of PCN congeners (as % of ∑ 26 PCN) in African countries

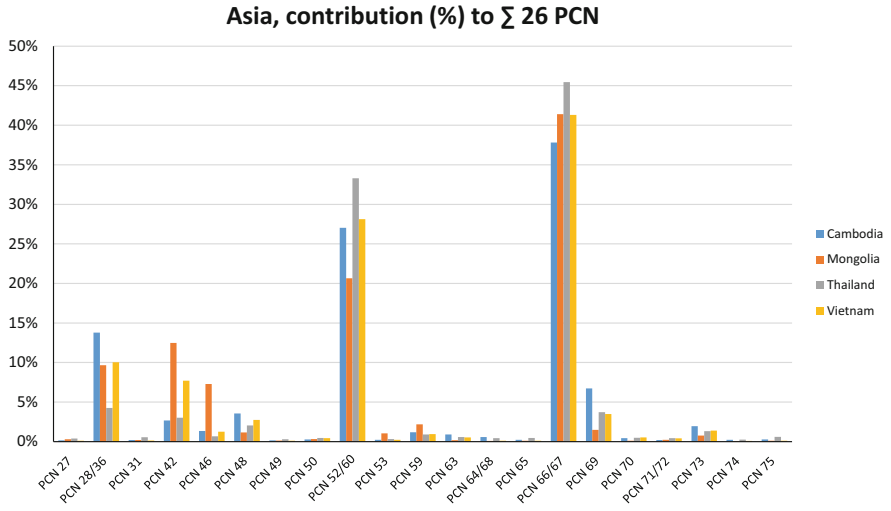

Fig. 6 Pattern of PCN congeners (as % of ∑ 26 PCN) in Asian countries

Overall, the combined global data from these human milk WHO surveys indicate that PCN showing dioxin-like toxicity represent a significant part of the total amount of PCN in this matrix. Especially, 1,2,3,4,6,7-HexaCN/1,2,3,5,6,7-HexaCN (PCN 66/67) represents approximately 20–40% of the total amount of PCN. 2,3,6,7-TetraCN (PCN 48), a congener with lateral substitution like 2,3,7,8-TCDD, however smaller size and therefore lower AhR binding potency, is generally present between 1 and 4% of the total.

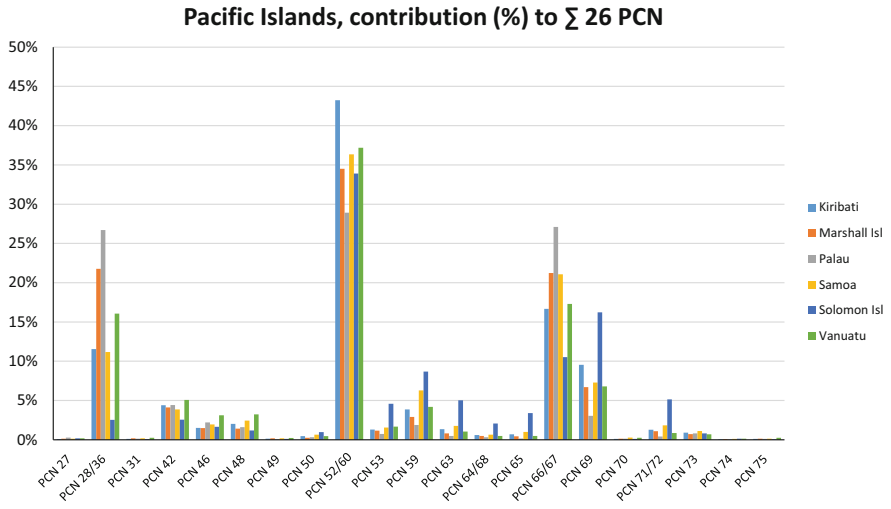

Fig. 7 Pattern of PCN congeners (as % of ∑ 26 PCN) in countries from the Pacific Islands

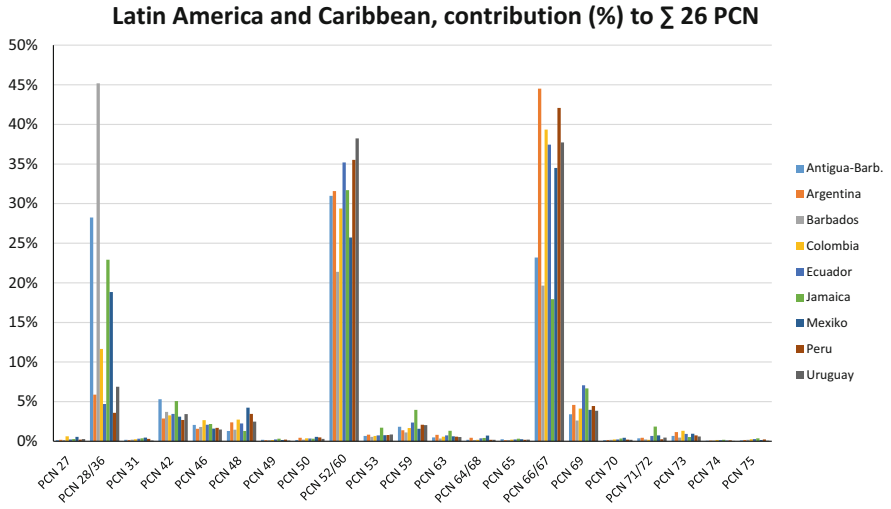

Fig. 8 Pattern of PCN congeners (as % of ∑ 26 PCN) in Latin American and Caribbean countries

There may be other PCN congeners of toxicological interest such as 1,2,3,6,7-PentaCN (PCN 54) that was not included in the analysis. This congener resembles 1,2,3,7,8-PentaCDD that shows potent dioxin-like activity as described in Sect. 1 (introduction). PCN 54 was absent or found at extremely low concentrations in PCN technical mixtures (Hanari et al. 2013). A mass percent contribution of 0.05% in Halowax 1014 was found (Helm et al. 1999). It has not appeared to show significant presence in human serum samples from an industrial city in Eastern China when

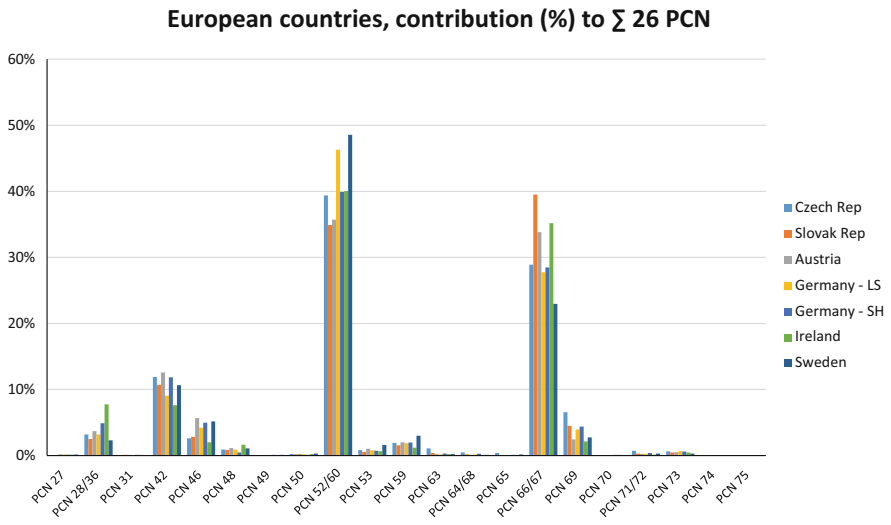

Fig. 9 Pattern of PCN congeners (as % of ∑ 26 PCN) in European countries

industrial technical products and industrial thermal processes are seen as a relevant pathway to human exposure (Jin et al. 2019). It was found in low concentrations when combustion related sources are dominant as observed in a study on pine needles across Poland, where the PCN 54 contribution was about 0.2% of the total PCN (as median; for comparison, the median of the PCN 66/67 contribution: 0.6%) (Orlikowska et al. 2009). These findings might have implications depending on possible regional PCN sources (e.g., technical mixtures or combustion processes), and it would be informative to know if PCN 54 actually occurs in human milk. Thus, for completion of the database for human risk assessment, PCN 54 could be considered in future monitoring, as well.

Furthermore, the effect of metabolism of some dietary PCN congeners in humans and higher order animals (Fernandes et al. 2017) is likely to result in further modification of the occurrence profiles as seen in human milk. The abundance of PCN 52/60 and PCN 66/67 could indicate a significant role of metabolism as these congeners do not contain two adjacent hydrogen atoms, which is well known to facilitate metabolism of PCB, PCDD, and PCDF. However, the abundance could also arise from selective retention in vertebrates including humans, eventually resulting in substantially higher exposure to these specific congeners as seen in human fat and milk (Kunisue et al. 2009; Pratt et al. 2013).

3.3 Dioxin-Like Properties

Dioxin-like compounds (DLC) comprise polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and certain polychlorinated

biphenyls (PCB). Most of the most studied toxic and biological effects of these compounds are mediated through the aryl hydrocarbon receptor (AhR), a cytosolic receptor protein present in most vertebrate tissues. The AhR binds potently to a range of diverse compounds including contaminants such as 2,3,7,8–substituted PCDD/PCDF and some laterally substituted PCB. Different toxic potencies are expressed as Toxic Equivalency Factors (TEF) for DLC—a concept that allows for a combined expression of toxicity that represents all the relevant congeners that occur in a sample. Criteria for inclusion of a compound in the TEF concept are (1) structural similarity to PCDD/PCDF, (2) ability to bind to AhR; (3) ability to elicit AhR-mediated biochemical and toxic responses; (4) to be persistent and accumulate in the food chain (Van den Berg et al. 1998). The reported information on PCN shows an ability to meet all these criteria and there are a number of studies that report AhR binding and the relative potencies of this action (Engwall et al. 1994; Blankenship et al. 2000; Falandysz et al. 2014; Fernandes et al. 2017, 2022b). It is clear therefore that PCN should be considered in the TEF concept (Van den Berg et al. 2006; Fernandes et al. 2022b).

In terms of human exposure, the expression of toxicity-normalized concentrations for dietary PCN content, which mirrors the approach used for PCDD/PCDF and PCB, has a number of advantages. In general, a significant part of PCN toxicity is considered dioxin-like and therefore it allows an association and comparison with the toxic content arising from PCDD/PCDF and PCB. Moreover, it also allows a more holistic consideration of the total dioxin-like toxicity of, e.g., human milk (Fernandes et al. 2022b) as the effects of individual congeners are considered additive. Practically, for risk assessment and comparative purposes, the expression of the total dioxin-like toxicity as a single value (rather than a set of individual congener concentrations) allows more efficient handling and comparison of the data. In earlier periods, when there was very little occurrence data on PCN (or it was presented as homologue group concentrations) there was understandably a reluctance to apply a TEF approach to PCN. However, during the last two decades more data on the occurrence in food and human tissues has emerged including frequently individual congener concentrations.

Although the current volume of such studies may be insufficient to establish robust TEFs for PCN, relative potency values (REPs) can be used as an interim conservative approach for the cumulative risk assessment of PCN. The approach can also be used to determine the relative contribution of these PCN compared with PCDD/PCDF and PCB (COT 2009; Fernandes et al. 2010, 2017, 2022b).

3.3.1 Relative Potency of PCN

At present, there is one in vivo study available (Hooth et al. 2012). Therefore, most of these REPs were derived from in vitro studies. A review of in vitro and in silico REPs for selected individual PCN congeners derived from five studies (Hanberg et al. 1990; Blankenship et al. 2000; Villeneuve et al. 2000; Behnisch et al. 2003; Puzyn et al. 2007) shows a range of potencies (Fernandes et al. 2017) (Table 4). Another study on 42 PCN using DR-CALUX showed dioxin-like activities of

Table 4 Reported ranges of combined in vitro and in silico relative potencies (REPs) for selected individual PCN congeners (reprinted from Fernandes et al. 2017 with permission from Elsevier)

Configuration	PCN #	*In vitro* REPs						*In silico* REPs	
		H4II-EROD	H4II-EROD	H4II-luc		DR-CALUX-(pM)	Micro-EROD (pM)		
Source Reference →		Villeneuve et al., 2000	Hanberg et al., 1991	Blankenship et al., 1999, 2000		Behnish et al., 2003		Puzyn et al., 2007	
1,2,3,5,7-PeCN	**52**	4.2×10^{-6}				*< 3.4 x 10^{-6}*	*< 1.8 x 10^{-6}*	8.5×10^{-6}	3.8×10^{-5}
1,2,3,5,8-PeCN	**53**					*< 1.8 x 10^{-6}*	*< 1.2 x 10^{-6}*	1.3×10^{-8}	5.2×10^{-6}
1,2,3,6,7-PeCN	**54**	9.2×10^{-5}		< 0.00069	0.00017	0.00058		2.8×10^{-5}	5.5×10^{-5}
1,2,3,7,8-PeCN	**56**	2.4×10^{-5}		0.00049				2.3×10^{-5}	5.6×10^{-5}
1,2,4,6,7-PeCN	**60**	$< 4.2 \times 10^{-7}$			$< 2.8 \times 10^{-5}$			1.3×10^{-6}	2.8×10^{-5}
1,2,3,4,5,6-HxCN	**63**		0.002					2.2×10^{-5}	2.2×10^{-5}
1,2,3,4,5,7-HxCN	**64**		2×10^{-5}					1.1×10^{-4}	1.0×10^{-5}
1,2,3,4,6,7-HxCN	**66**	0.00061		0.0024	0.0039	0.0012	0.00054	0.00069	0.0029
1,2,3,5,6,7-HxCN	**67**	0.00028	0.002		0.001	0.00048		0.001	0.0017
1,2,3,5,6,8-HxCN	**68**		0.002		0.00015	0.00049		0.00027	0.00011
1,2,3,5,7,8-HxCN	**69**		0.002			1.1×10^{-4}	6.4×10^{-6}	8.3×10^{-7}	1.5×10^{-4}
1,2,3,6,7,8-HxCN	**70**	0.0021		0.0095	0.00059	0.0028		0.0028	0.00071
1,2,4,5,6,8-HxCN	**71**					*< 1.1 x 10^{-6}*		4.3×10^{-5}	1.6×10^{-7}
1,2,4,5,7,8-HxCN	**72**					6.0×10^{-5}	*7.1 x 10^{-6}*	1.0×10^{-4}	8.9×10^{-8}
1,2,3,4,5,6,7-HpCN	**73**	0.0004	0.003	0.0006	0.001	0.00052		0.00038	0.0018
1,2,3,4,5,6,8-HpCN	**74**					*4.1 x 10^{-6}*			1.0×10^{-7}
1,2,3,4,5,6,7,8-OCN	**75**					1.0×10^{-5}	*< 4.3 x 10^{-6}*		

31 congeners. REPs determined in previous studies were comparable to REP values obtained in this study (Suzuki et al. 2020).

The choice of congeners covered in this study was based principally on the toxicological characteristics of individual PCN congeners and the levels and patterns of their occurrence in foods. Thus, this study included tetra- to octa-chlorinated compounds, and generally those that were reported to show the highest REP values, e.g., PCN 66, 67, 70, etc. In order to provide an indication of the dioxin-like toxicity arising from the presence of PCN, the data were calculated as toxic equivalents (TEQ), computed using REPs (1) used for human exposure studies (Falandysz et al. 2019; 2020; Fernandes et al. 2010, 2011, 2017, 2022b; Pratt et al. 2013; Zhihua et al. 2019; Zacs et al. 2021) and (2) other suggested REPs (Falandysz et al. 2014), which were derived as reasonable approximation with regard to the wide range of in vitro and in silico REPs as listed in Table 4 and the in vivo REPs for PCN 66 and 67 (Hooth et al. 2012) (Table 5).

As these REP for PCN have been derived from mostly in vitro studies, the question arises as to which extent these REPs are actually realistic for in vivo conditions, especially in those situations when prolonged exposure takes places, e.g., a breastfed infant. At present only one in vivo study has been done which

Table 5 Relative potencies (REPs) (1) used in human exposure studies; (2) other suggested REPs; (3) REPs derived from the in vivo study

Configuration	PCN congener	REPs used in human exposure studies*)	Other suggested REP values **)	REPs derived from in vivo study ***)
1,2,3,4-TeCN	27	0.00000023		
1,2,4,7-TeCN	34			
1,2,5,6-TeCN	36	0.00000021		
1,2,6,7-TeCN	39			
1,2,6,8-TeCN	40	*		
1,3,5,7-TeCN	42			
2,3,6,7-TeCN	48	0.000024		
1,2,3,4,5-PeCN	49	0.000033		
1,2,3,4,6-PeCN	50	0.0001		
1,2,3,5,7-PeCN	52	0.000025		
1,2,3,5,8-PeCN	53	0.0000018		
1,2,3,6,7-PeCN	54	*	0.0002	
1,2,3,7,8-PeCN	56	*	0.000005	
1,2,4,5,6-PeCN	57		0.000001	
1,2,4,6,7-PeCN	60	0.0001	0.000001	
1,2,3,4,5,6-HxCN	63	0.00017	0.00002	
1,2,3,4,5,7-HxCN	64	0.0028	0.00001	
1,2,3,4,6,7-HxCN	66	0.004	0.002	0.0015–0.0072
1,2,3,5,6,7-HxCN	67	0.004	0.002	0.00029–0.00067
1,2,3,5,6,8-HxCN	68	0.0028	0.0005	
1,2,3,5,7,8-HxCN	69	0.002	0.0001	
1,2,3,6,7,8-HxCN	70	0.0051	0.003	
1,2,4,5,6,8-HxCN	71	0.00009	0.00001	
1,2,4,5,7,8-HxCN	72	0.00009		
1,2,3,4,5,6,7-HpCN	73	0.0031	0.0006	
1,2,3,4,5,6,8-HpCN	74	0.0000041		
1,2,3,4,5,6,7,8-OCN	75	0.00001	0.00001	

Source references: *) Falandysz et al. (2019), Fernandes et al. (2010, 2011, 2017, 2022b), Zhihua et al. (2019), Zacs et al. (2021), **) Falandysz et al. (2014), ***) Hooth et al. (2012)

included direct comparison with 2,3,7,8-TCDD and covered biological as well as toxicological endpoints after a two-week oral exposure (Hooth et al. 2012). This study included both PCN 66 and 67 that both are structural analogs with 1,2,3,4,7,8 and 1,2,3,6,7,8-HxCDD that both have a relatively high WHO TEF of 0.1 (Van den Berg et al. 2006). In view of the high contribution of PCN 66 and 67 to the total amount of PCN, it is relevant to determine if the REPs based on in vitro experiments and listed in Table 5 are close to or similar to those obtained in vivo. The in vivo

study with PCN 66 and 67 determined REPs of, respectively, 0.0015–0.0072 and 0.00029–0.00067 for CYP1A1, CYP1A2, and thymic atrophy.

Both sets of REPs, those used in human exposure studies and those proposed by Falandysz et al. (2014) were used for the TEQ calculations of the WHO/UNEP human milk samples. For PCN 66, the REPs of 0.004, respectively, 0.002 are in comparison with the results from the in vivo study in good agreement. However, the REP for PCN 67 used in human exposure studies is approximately one order of magnitude higher than that obtained in the in vivo study (Hooth et al. 2012), the proposed REP half of that.

In vitro REPs for PCN may overestimate the actual in vivo effect which is in line with similar comparisons made for REPs of some PCDD/PCDF and dl-PCB. The most likely reason for this discrepancy is caused by the lack of metabolism in vitro (Van den Berg et al. 1998, 2006). This may lead to overestimation of the calculated PCN-TEQ in human milk depending on the extent of the contribution of PCN 67 to the summed PCN-TEQ.

Therefore, the individual contribution of PCN 66 and PCN 67 to TEQ could be calculated by use of different REPs for these two congeners with adjustments for PCN 67 closer to the range determined in the in vivo study, if the individual concentrations were known. However, a chromatographic separation of PCN 66 and 67 is not possible under routine GC conditions, and these two congeners can be determined routinely only as the sum of the two congeners (see Subsection 3.3.3 in the following). The relative proportions of PCN 66 and PCN 67 in the milk samples are therefore not known, so the precautionary principle was applied, using the higher REP to allow a higher margin of safety, as seen similarly in other PCN studies on food and human milk (Pratt et al. 2013; Fernandes et al. 2017; Zacs et al. 2021). While this is conservative, it may also be a health protective approach. Therefore, for the sum parameter of the co-eluting pair, the REP applied to PCN 66 is also applied to PCN 67.

As conclusion, in addition to the discussion of scientifically sound REPs for these congeners, there is the analytical issue of determining individual concentrations for PCN 66 and 67 in samples. Only a special GC column with considerable limitations under routine conditions could separate PCN 66 and 67 (Helm 2002; see Subsection 3.3.3 in the following) and was used for determination of the mass percent contribution of these congeners in Halowax 1014: PCN 66 contributed 0.47%, PCN 67 0.29% (determined as single congeners), resulting in a ratio of 1.62 to 1. Determined by negative ion mass spectrometry, the peak area ratio in Halowax 1014 was 1.87, in Halowax 1013 1.43 and in Halowax 1099 1.36, in air of the Lake Ontario 1.3 (Helm et al. 1999). Thus, about 55% to 60% of the sum of PCN 66 and 67 came from PCN 66. According to our best of knowledge, these are the only available quantitative data for the ratio between PCN 66 and 67. Both congeners do not contain two adjacent hydrogen atoms, which is well known to facilitate metabolism in PCB, PCDD, and PCDF. Therefore, it can be assumed that the ratio is not changed due to metabolic processes during bioaccumulation to human matrices. As a result with regard to the first step (determination of individual concentrations) for a more precise estimation with the aim of a congener-specific assessment, it could be assumed that

about 55% to 60% of the PCN 66/67 concentration comes from PCN 66. Then, as second step, these estimated individual concentrations could be multiplied by the REPs for PCN 66 as used in human exposure studies or as proposed by Falandysz et al. (2014), and by an REP of 0.0005 for PCN 67, which is in close agreement with the in vivo study. For more details and comparison of results of different approaches, see Subsection 3.3.3.

These PCN-TEQ values provide an interim indication of the PCN-associated dioxin-like toxicity in the human milk samples of the 2016–2019 study. To the best of our knowledge these analyses of PCN in human milk and their estimated contribution to dioxin-like toxicity are the first study covering this aspect so thoroughly.

3.3.2 Calculation of PCN-TEQ, Part 1: Lower–Upper Bound Results; Co-Eluting Congeners PCN 52/60 and 64/68

Different imputation approaches for handling "non-detects" (more exactly: not quantified congeners) in PCDD/PCDF analysis were tested using the limit of detection (LOD), among them: (1) calculation of the contribution of each non-detected congener to the TEQ as zero (lower bound concentrations); (2) calculation of the contribution of each non-detected congener to the TEQ as the limit of detection (upper bound concentrations); (3) calculation of the contribution of each non-detected congener to the TEQ as half of the limit of detection (middle bound concentrations) (Hoogerbrugge and Liem 2000). Later, these proposed definitions of lower and upper bound concentrations were used as pillars of the analytical criteria, but they were based on the limit of quantification (LOQ) rather than the LOD: An important criterion for assessing the reliability of estimated TEQ that are derived from PCDD/PCDF and dioxin-like PCB concentrations is the difference between the lower bound and upper bound TEQ result (Malisch et al. 2023b, Malisch and Schächtele 2023). As a measure of satisfactory analytical quality assurance and quality control (QA/QC), this difference should be less than 20% (UNEP 2019).

The median of the differences between lower and upper bound PCN-TEQ in all 40 samples was 0.3%. The range was between 0% to 2%, if REPs as used for human biomonitoring were applied, and between 0% and 3%, if other suggested REPs were used (Table 6). Therefore, all samples analyzed in this study fulfilled this QA/QC criterion. These differences were considered negligible. Thus, as for PCDD/PCDF and dioxin-like PCB, only the upper bound PCN-TEQ are used for discussion of the results.

With regard to the REPs used in human exposure studies and the other suggested REPs as listed in Table 5, two co-eluting pairs had different REPs for the individual PCN: (1) PCN 52/60 applying REPs used in human biomonitoring; (2) PCN 64/68 using other suggested REPs. The results discussed in this article are based on the assumption that, where congeners co-eluted, the content comprised only the congener with the higher REP. Use of the higher REP for the co-eluting congeners increases the PCN-TEQ by 2.1% as median (range 0.9% to 3.6%), if 0.0001 for PCN 60 is used instead of 0.000025 for PCN 52; by 0.3% as median

Table 6 Comparison of lower bound (LB) and upper bound (UB) PCN-TEQ concentrations (pg PCN-TEQ/g lipid), calculated by use of (1) REPs used in human biomonitoring and (2) other suggested REPs and differentiated by use of the lower and higher REP for individual PCN in cases of co-eluting congeners

	Median	Min	Max
Human biomonitoring REPs, here for PCN 52/60 with lower REP as for PCN-52: 0.000025			
PCN-TEQ (LB)	0.073	0.026	0.226
PCN-TEQ (UB)	0.073	0.026	0.227
Difference PCN-TEQ UB-LB (%)	0.3%	0.0%	2.0%
Human biomonitoring REPs, here for PCN 52/60 with higher TEF as for PCN 60: 0.0001			
PCN-TEQ (LB)	0.074	0.026	0.231
PCN-TEQ (UB)	0.074	0.027	0.232
Difference PCN-TEQ UB-LB (%)	0.3%	0.0%	1.9%
PCN-TEQ with REP for PCN 60 as % of PCN-TEQ with REP for PCN 52	102.1%	100.9%	103.6%
Other suggested REPs, here for PCN 64/68 with lower REP as for PCN 64: 0.00001			
PCN-TEQ (LB)	0.029	0.010	0.100
PCN-TEQ (UB)	0.029	0.010	0.100
Difference PCN-TEQ UB-LB (%)	0.3%	0.0%	3.0%
Other suggested REPs, here for PCN 64/68 with higher REP as for PCN 68: 0.0005			
PCN-TEQ (LB)	0.029	0.010	0.100
PCN-TEQ (UB)	0.029	0.010	0.101
Difference PCN-TEQ UB-LB (%)	0.2%	0.0%	2.9%
PCN-TEQ with REP for PCN 60 as % of PCN-TEQ with REP for PCN 52	100.3%	100.1%	104.3%

(range 0.1% to 4.3%), if 0.0005 is used for PCN 68 instead of 0.00001 for PCN 64 (Table 6). Also these differences are considered negligible.

3.3.3 Calculation of PCN-TEQ, Part 2: Co-Eluting Congeners PCN 66/67

A problem for calculation of the individual contribution of PCN 66 and 67 to PCN-TEQ is the lack of the individual concentrations for these congeners. Chromatographic separation of PCN 66 and 67 is not possible under routine GC conditions: For this study, six different kinds of GC columns as commonly used for confirmation analyses of pesticides or contaminants were not able to separate this co-eluting pair. Therefore, for routine analysis only the sum of the two congeners was determined. This was also the only determinable parameter for PCN 66 and 67 in an interlaboratory study on PCN performed by the EU Reference Laboratory for POPs with 10 laboratories participating in 2021 (Schächtele et al. 2023).

Nine different GC columns were checked for separation of otherwise co-eluting PCB and PCN congeners. The Rxi-17SilMS demonstrated the most drastic difference in PCB selectivity and, to a lesser extent, PCN when compared with the other eight columns and could work as a confirmatory column or as a second dimension column for GC × GC separations. However, the very close retention times

(41.79 min for PCN 66, 41.81 min for PCN 67) show that in practice these two congeners could not be separated (Stultz and Dorman 2020).

Only a special GC column (Rt-βDEXcs) could separate PCN 66 and 67, however, with considerable limitations under routine conditions (long GC run times of about 100 min, high column bleeding and therefore increased maintenance of the MS ion source) (Helm 2002). This column was used for determination of the mass percent contribution of these congeners in technical products and air (Helm et al. 1999). Furthermore, this special GC column was used for separation of closely eluting PCN congeners including PCN 66 and 67 in technical mixtures by two-dimensional GC/quadrupole mass spectrometric detection (GC × GC/qMS) on Rt-βDEXcst and DB-Wax phases. However, no quantitative data were given on the composition, and neither PCN 66 nor 67 was shown in the figures on relative abundance (Hanari et al. 2013). As a conclusion, the determination of the concentration of the individual congeners PCN 66 and 67 in human milk is not possible under routine conditions and requires research for development of a practical method with sufficient sensitivity and its validation. Therefore, as derived above (Subsection 3.3.1) the assumption that about 55% to 60% of the PCN 66/67 concentration comes from PCN 66 is presently the most practical option and was used for estimation of specific PCN 66 and 67 concentrations, to be multiplied by the respective REPs for calculation of the separate contributions to PCN-TEQ. Then, 0.0005 was used as REP for PCN 67 in good agreement with the in vivo study.

As a result, the PCN-TEQ calculated by the two sets of REPs (used in human exposure studies with identical REPs for PCN 66 and 67 [0.004 for both congeners] or those proposed by Falandysz et al. 2014 also with identical REPs for PCN 66 and 67 [0.002 for both congeners]) can be compared with the PCN-TEQ based on these specifically estimated contributions of PCN 66 and 67.

Furthermore, a kind of «middle bound» TEQ can be calculated. The lower/middle/upper bound concept for TEQ calculations (see Subsection 3.3.2) was developed for handling of «non-detects» and thus a question related to the analytical sensitivity and the LOQ. As conservative approach and for protection of the consumer, maximum levels for TEQ deriving from PCDD/PCDF and dioxin-like PCB are based on upper bound concentrations, and the analytical criteria with acceptable differences between lower and upper bound results below 20% make sure that reliable analytical results are obtained. In contrast, at times before introduction of these criteria, relatively large differences between lower and upper bound values were seen. Then, for risk assessment, the application of the upper bound concentrations leads to an overestimation of the intake, the application of the lower bound concentrations to an underestimation of the intake. For these cases, the application of a «middle bound» TEQ concentration was recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Canady et al. 2002). However, the PCN 66/67 issue and possible consequences for over- or underestimation of TEQ is not a question of handling of LOQs (all results were well above LOQ), but of use of too high or too low REPs. Therefore, in analogy, a «middle bound» TEQ was calculated as mean of the lower and upper range for both standard procedures (applying REPs used in human exposure studies or those

Table 7 PCN-TEQ concentrations (pg PCN-TEQ/g lipid) calculated by use of (1) REPs used in human exposure studies and (2) other suggested REPs (Falandysz et al. 2014) comparing the standard method, the "middle bound approach" and the congener-specific estimations for PCN 66 and 67

PCN-TEQ calculated by	Median	Min	90% Quantile	Max
REPs used in human exposure studies				
Standard method	0.074	0.027	0.172	0.232
"Middle bound approach"	0.070	0.025	0.162	0.219
Congener-specific estimations for PCN 66 and 67	0.051	0.020	0.113	0.159
Other suggested REPs (Falandysz et al. 2014)				
Standard method	0.029	0.010	0.080	0.101
"Middle bound approach"	0.054	0.018	0.149	0.186
Congener-specific estimations for PCN 66 and 67	0.021	0.007	0.055	0.069

proposed by Falandysz et al. 2014) by multiplying the sum parameter PCN 66/67 (1) with the lowest REP derived from in vivo studies (0.00029) for the lower range, and (2) with the highest REP derived from in vivo studies (0.0072) for the upper range.

Table 7 shows the results of these six approaches to calculate PCN-TEQ. The key question was whether the PCN-TEQ calculated by the standard methods would be too high and their contribution to the total amount of TEQ in human milk overestimated, depending on the extent of the contribution of PCN 67 to PCN-TEQ as a result of its concentration and the applied REP. If for calculation of PCN-TEQ the REPs used in human exposure studies are applied, the results of the «middle bound approach» are similar to the standard method. The PCN-TEQ based on the congener-specific estimations are about 30% lower than the standard method. PCN-TEQ calculated by REPs as suggested by Falandysz et al. (2014) are about 60% lower than by REPs used in human exposure studies. The PCN-TEQ based on the congener-specific estimations are again about 30% lower than the standard method. The «middle bound approach» leads to higher PCN-TEQ values, closer to the values based on REPs used in human exposure studies.

As a conclusion, harmonization of the value of the REPs used is more important than the separate determination of PCN 66 and 67. PCN-TEQ based on the two sets of REPs differ by a factor of 2.2 (as median of the factors for the individual country results obtained by the standard method), whereas the congener-specific determination is expected to result in differences of approximately 30% to the standard method. These uncertainties can only be reduced by (1) assessment of the wide range of reported REPs and assignment of one set of proposed REPs or TEFs, at least for PCN 66 and 67 as most important congeners, and (2) analytical research and method development allowing the determination of PCN 66 and 67 in human milk samples separately.

3.4 PCN-TEQ Results

The median PCN-TEQ concentration in 40 pooled human milk samples from 39 countries was 0.07 pg PCN-TEQ/g lipid (range 0.03 pg/g to 0.23 pg/g), if calculated with the REPs as applied in human biomonitoring studies, and 0.03 pg PCN-TEQ/g lipid (range 0.01 pg/g to 0.10 pg/g), if calculated with other suggested REPs. Human milk from European countries had considerably higher TEQ levels than those found in the African region, the Asia-Pacific region, and the Latin American and Caribbean region (Table 8). Figures 10, 11, 12, and 13 illustrate the findings in countries of these regions. In all regions and all countries, the PCN-TEQ concentrations were higher by a factor of about 2.2 when calculated by REPs as applied in human biomonitoring studies in comparison with other suggested REPs.

Table 8 Range of PCN-TEQ concentrations (pg PCN-TEQ/g lipid) in the African region, the Asia-Pacific region, the Latin American and Caribbean region, and European countries calculated by (1) REPs as used in human biomonitoring studies and (2) other suggested REPs (Falandysz et al. 2014)

	Human biomonitoring REPs			Other suggested REPs		
PCN-TEQ	Median	Min	Max	Median	Min	Max
Africa	0.07	0.03	0.10	0.03	0.01	0.04
Asia-Pacific	0.06	0.03	0.12	0.03	0.01	0.06
Latin America and Caribbean	0.05	0.04	0.11	0.03	0.02	0.05
European countries	0.20	0.13	0.23	0.09	0.06	0.10

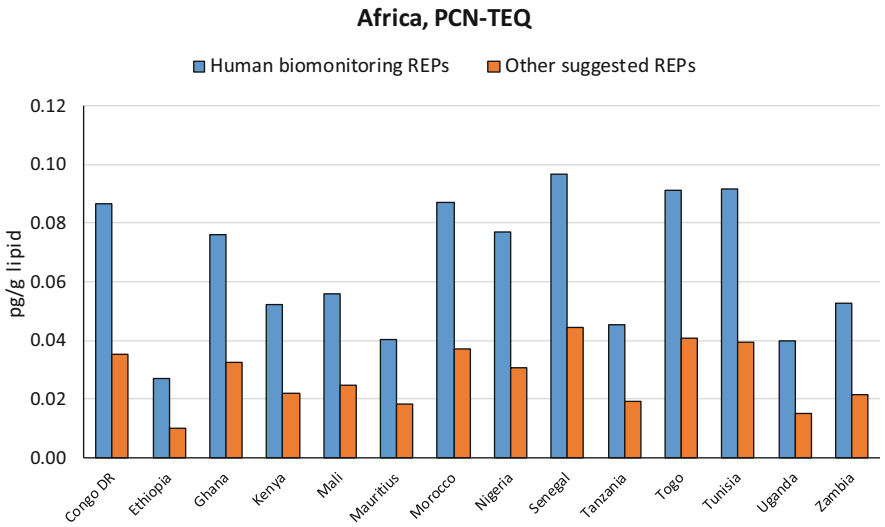

Fig. 10 Results of the 2016–2019 survey for PCN-TEQ concentrations in human milk in countries from Africa (pg PCN-TEQ/g lipid), if calculated with (1) REPs as used in human biomonitoring studies and (2) other suggested REPs (Falandysz et al. 2014)

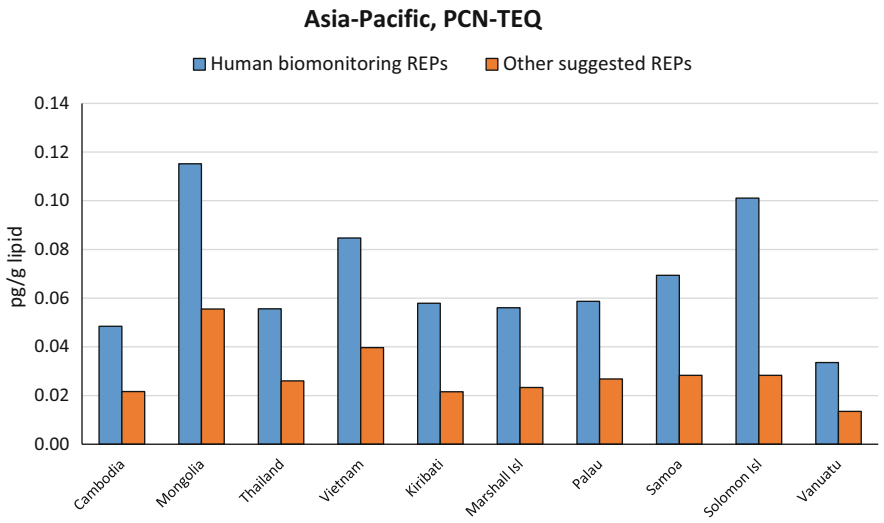

Fig. 11 Results of the 2016–2019 survey for PCN-TEQ concentrations in human milk in countries from the Asia-Pacific region (pg PCN-TEQ/g lipid), if calculated with (1) REPs as used in human biomonitoring studies and (2) other suggested REPs (Falandysz et al. 2014)

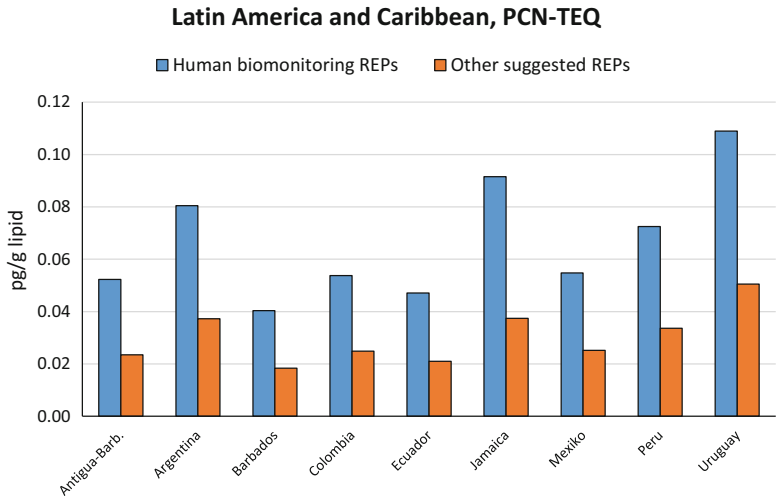

Fig. 12 Results of the 2016–2019 survey for PCN-TEQ concentrations in human milk in countries from Latin American and Caribbean countries (pg PCN-TEQ/g lipid), if calculated with (1) REPs as used in human biomonitoring studies and (2) other suggested REPs (Falandysz et al. 2014)

Among the PCN congeners included in this study, by far the highest contribution (90%) to the dioxin-like toxicity was attributed to PCN 66/67. This contribution is a result of relatively high concentrations within the group of 26 PCN (see Subsection 3.2 «PCN patterns») combined with the relatively high REPs used, notwithstanding

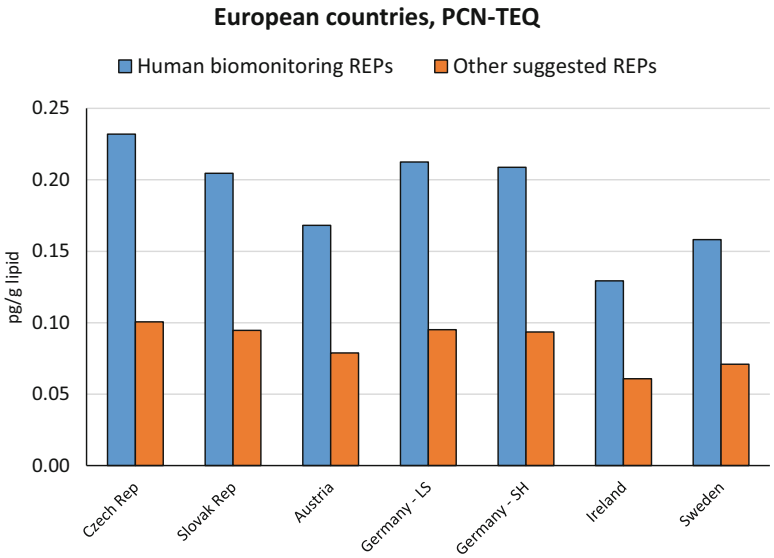

Fig. 13 Results of the 2016–2019 survey for PCN-TEQ concentrations in human milk in countries from European countries (pg PCN-TEQ/g lipid), if calculated with (1) REPs as used in human biomonitoring studies and (2) other suggested REPs (Falandysz et al. 2014)

whether the values used were those taken from the human biomonitoring studies or from other suggested REPs (Falandysz et al. 2014). Differences between these two REP groups for PCN 69 result in differences between the contribution to PCN-TEQ (median 7%, maximum 37%, if REPs used for human biomonitoring studies are applied; median 1%, maximum 7%, if the other suggested REPs are applied). Although PCN 52/60 occurs at relatively high levels in all samples the low REP value results in a contribution of about 3% (range 1% to 5%) to PCN-TEQ, when REPs used for human biomonitoring studies are applied, and about 0.1%, if other suggested REPs (Falandysz et al. 2014) are applied. Other congeners with minor TEQ contributions were PCN 64/68, PCN 70, and PCN 73 (Figs. 14 and 15). As a result, the assessment of PCN 66 and PCN 67 in order to obtain confirmed TEF would be most important for calculations of the dioxin-like toxicity of PCN, followed by PCN 69.

3.5 Contribution of PCN-TEQ to the Cumulative TEQ (Including the Overall Sum of PCDD, PCDF, and Dioxin-like PCB Toxic Equivalents [WHO_{2005}-TEQ])

Finally, the contribution of PCN to the cumulative TEQ (including the overall sum of toxic equivalents with PCDD, PCDF, and dioxin-like PCB [WHO-PCDD/PCDF-PCB-TEQ = WHO_{2005}-TEQ, see Malisch et al. 2023b]) is of interest. In Subsection 3.3.1, the problem to determine exactly the contribution of PCN 66 and 67 to

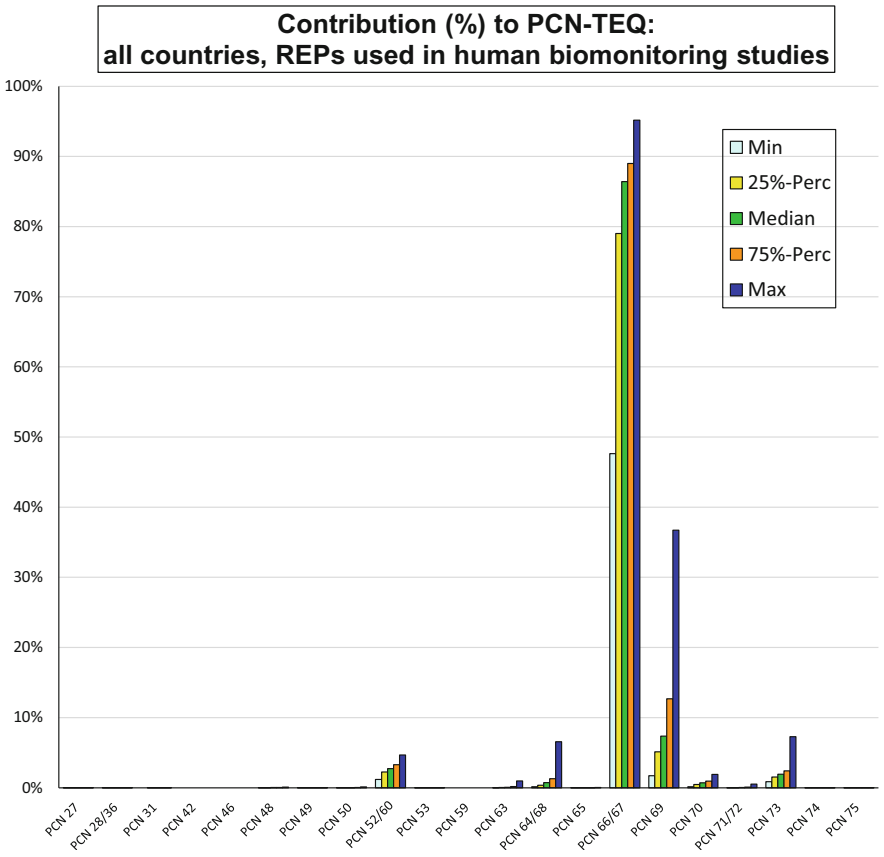

Fig. 14 Contribution (%) of PCN congeners to PCN-TEQ calculated with REPs used in human biomonitoring studies

PCN-TEQ was discussed, and in Subsection 3.3.3 options to answer the key question whether the PCN-TEQ calculated by the standard methods would be too high and their contribution to the total amount of TEQ in human milk overestimated, depending on the extent of the contribution of PCN 67 to PCN-TEQ as a result of its concentration and the applied REP. The results of the six approaches discussed in Subsection 3.3.3 were assessed for their contribution to the cumulative TEQ as sum of WHO_{2005}-TEQ and PCN-TEQ. On average, the contribution of PCN-TEQ to the cumulative TEQ is between 1% and 2% calculated by these six approaches, with a wider range up to 5% for the 39 countries of this study within each of these approaches (Table 9).

This is about an order of magnitude lower than the contribution of dioxin-like PCB to the cumulated TEQ (median 26.4%, range 8.1% to 54.9%). These differences between the contribution of PCN and DL-PCB reflect the differences

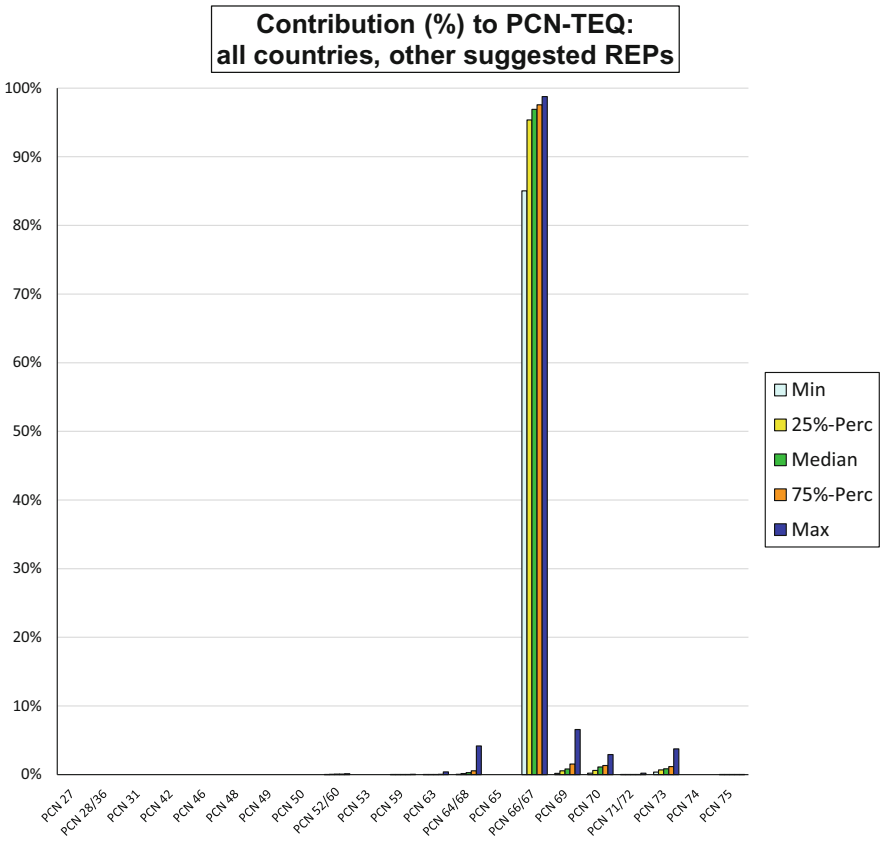

Fig. 15 Contribution (%) of PCN congeners to PCN-TEQ calculated with other suggested REPs (Falandysz et al. 2014)

between estimated production rates between PCN (estimated range 150,000 to 400,000 tons (Falandysz 1998, Falandysz and Fernandes 2020)) and PCB (more than one million tons of technical PCB mixtures (Breivik et al. 2002; EFSA 2005)).

PCN-TEQ calculated by the standard method with REPs used in human biomonitoring studies add on average in Africa, the Asia-Pacific region, and Latin American and Caribbean countries, about 2% to the cumulative TEQ, and in European countries about 4%. If results are calculated by the standard method using the other suggested REPs (Falandysz et al. 2014), the average contribution in Africa, the Asia-Pacific region, and Latin American and Caribbean countries is about 1% and in European countries about 2% (Table 10).

Table 9 Range of contribution of PCN-TEQ to the cumulated TEQ (as sum of toxic equivalents with PCDD, PCDF, and dioxin-like PCB [WHO-PCDD/PCDF-PCB-TEQ = WHO_{2005}-TEQ] and PCN-TEQ) in human milk calculated by six different approaches for determination of PCN-TEQ for 40 pooled samples from 39 countries of the 2016–2019 study

% contribution to cumulated TEQ calculated by	Median	Min	90% Quantile	Max
REPs used in human exposure studies for PCN-TEQ				
Standard method	2.0%	0.5%	3.9%	4.7%
"Middle bound approach"	1.9%	0.5%	3.8%	4.5%
Congener-specific estimations for PCN 66 and 67	1.4%	0.3%	2.8%	3.3%
Other suggested REPs for PCN-TEQ (Falandysz et al. 2014)				
Standard method	0.9%	0.2%	1.7%	2.3%
"Middle bound approach"	1.6%	0.4%	3.0%	4.1%
Congener-specific estimations for PCN 66 and 67	0.6%	0.1%	1.1%	1.6%
DL-PCB as % of cumulated TEQ	26.4%	8.1%	39.6%	54.9%

Table 10 Range of contribution of PCN-TEQ to the cumulative TEQ (as sum of toxic equivalents with PCDD, PCDF, and dioxin-like PCB [WHO-PCDD/PCDF-PCB-TEQ = WHO_{2005}-TEQ] and PCN-TQ) in the African region, the Asia-Pacific region, the Latin American and Caribbean region, and European countries

% PCN-TEQ (UB) as % of cumulative TEQ	Human biomonitoring REPs			Other suggested REPs		
	Median	Min	Max	Median	Min	Max
Africa	2.0%	0.8%	2.8%	0.8%	0.3%	1.2%
Asia-Pacific	1.9%	0.5%	3.9%	0.8%	0.2%	1.5%
Latin America and Caribbean	1.8%	1.0%	2.1%	0.8%	0.5%	1.0%
European countries	4.1%	2.7	4.7%	1.9%	1.3%	2.3%

Acknowledgments This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk studies, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway, and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure studies for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the WHO/UNEP Reference Laboratories, which included great efforts to plan and implement the national studies with the assistance of the health,

environment, laboratory, and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the Reference Laboratories was an important aspect for the successful organization of these studies on a global level.

We also thank the team at CVUA Freiburg for the performance of the analyses of the POPs presented in this publication.

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Part IV

Assessments

Time Trends in Human Milk Derived from WHO- and UNEP-Coordinated Exposure Studies, Chapter 1: Polychlorinated Biphenyls, Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans

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Abstract

Temporal trends of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in human milk were assessed by consideration only of countries with repeated participation in WHO/UNEP-coordinated exposure studies performed between 1987 and 2019. In contrast to a general estimation of time trends from all participating countries, this is a more precise approach because levels among countries are often highly variable. Studies on time trends for contaminants in human milk are important

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_12

components of the effectiveness evaluation of the Stockholm Convention on Persistent Organic Pollutants (POPs). There is no stipulation of a quantitative goal for the rate of reduction/decrease in POPs levels, however, as a quantitative objective for studies, these should have the ability to detect a 50% decrease in the levels of POPs within a 10-year period.

For non-dioxin-like PCB (calculated as the sum of six indicator PCB), a decrease of about 50% to 60% over 10 years was achieved in most of the five UN Regional Groups. Considerable decreases in concentrations with reductions of up to 95% over three decades were observed in European countries with high concentrations at the end of the 1980s, compared to slower decreases in less polluted ones. For the toxic equivalents (TEQ) of PCDD/PCDF and the total TEQ of PCDD/PCDF and dioxin-like PCB, a decrease of about 50% over 10 years was found mainly in Western European and some other countries with initially relatively high concentrations. TEQ concentrations of PCDD/PCDF decreased by up to 90% over three decades. Lower decreases observed in many countries have to be seen in the context of the quite low levels in these countries in comparison with other countries.

Keywords

Time trends · Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · Non-dioxin-like PCB · Dioxin-like PCB · PCDD/PCDF · Dioxins · Global WHO/UNEP studies · UN Regional Groups

1 Introduction

In this compendium, global human milk surveys for chemicals of health and environmental concerns are reviewed. In five parts, specific papers address various aspects: Part I gives a review of human milk surveys on persistent organic pollutants (POPs) from a historical perspective (Fürst 2023), an overview of the seven exposure studies coordinated by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) performed between 1987 and 2019 (Malisch et al. 2023a) and a review on the Stockholm Convention and its implementation by regional and global monitoring reports (Šebková 2023). Part II presents analytical methods and their validation for determination of chlorinated and brominated POPs between 2000 and 2019, including for polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (Malisch and Schächtele 2023). In Part III the findings for chlorinated and brominated POPs in samples collected between 2000 and 2019 are presented in various formats, among them for PCB and PCDD/PCDF (Malisch et al. 2023b). Part IV presents two kinds of assessments: of risk–benefit analysis for the breast-fed infant from dioxin-like compounds (van den Berg et al. 2023) and of time trends derived from countries with repeated participation in the WHO- and UNEP-coordinated studies in three chapters for (1) PCB and PCDD/PCDF (in this chapter);

(2) for DDT, beta-HCH and HCB and (3) perfluorinated alkyl substances. The assessment of temporal trends for polybrominated diphenylethers (PBDE) is included in the discussion of findings for polybrominated substances in Part III (Schächtele et al. 2023). Part V presents conclusions and key messages of these surveys.

A review of scientific publications between 1995 and 2011 on the spatial and temporal trends of Stockholm Convention POPs in breast milk compiles data on PCB and PCDD/PCDF levels providing a global overview (Fång et al. 2015). Furthermore, the regional and global monitoring reports for the Global Monitoring Plan assess datasets in the core media—ambient air, human tissues (human breast milk or blood), and water for hydrophilic POPs, but also other media such as soil, biota, plants are used to support interpretation of observed levels and their trends (Šebková 2023). These reports are available at the homepage of the Stockholm Convention (>Implementation>Global Monitoring Plan>Monitoring Reports).

One of the objectives of the WHO/UNEP-coordinated exposure studies was to generate comparable and consistent monitoring data on the presence of these contaminants in order to identify trends in levels. Adopted by the parties to the Stockholm Convention on POPs, *The Guidance Document on the Global Monitoring Plan* (GMP) considers such data on the presence of POPs in the environment and in human tissues necessary for the evaluation of the effectiveness of the Convention to eliminate or reduce emissions of specific POPs as required under Article 16 of the Convention. To provide reliable monitoring information for the Parties to the Stockholm Convention, as a quantitative objective for temporal studies the GMP guidance document proposed the ability to detect a 50% decrease in the levels of POPs within a 10-year period. This corresponds to an annual decrease of 7%. However, there is no stipulation of a quantitative goal for the rate of reduction/decrease in POPs levels. The Convention's objectives are either to eliminate or to reduce production, use and releases, depending on the annex where a chemical is listed, but the rate of decline is nowhere specified or required (UNEP 2015, 2019).

The presentation and discussion of the 2000–2019 results in Part III include **a first *general* estimation of time trends** for all 82 countries participating in these five rounds over these 20 years, comparing the temporal tendencies of the average concentrations of PCB and PCDD/PCDF in the studies over time (Malisch et al. 2023b). However, this chapter presents a more precise approach for the assessment of **temporal trends by consideration** of results only from those 50 of the 82 ***countries with repeated participation*** in the studies. Because levels correlate within countries more closely than among countries, this allows more certainty in drawing conclusions on time trends which are not potentially influenced by individual results from countries submitted just for a single round and seems optimal for the evaluation of the effectiveness for the purpose of Article 16 of the Convention. However, it should be noted that typically only very few time points from most individual countries are available which prevents from deriving statistically significant temporal trends in these cases. Yet, the existing data can indicate decreasing or increasing tendencies in POP concentrations. Furthermore, pooling of data in

regions allows to derive statistically significant time trends in the UN Regional Groups and globally. To minimize possible sources of variation for time trend analysis of POPs, including PCB, PCDD and PCDF, the concept of the WHO/UNEP-coordinated exposure studies has two basic elements: (1) preparation of pooled samples from a number of individual samples considered to be representative for a country or region/subgroup; (2) analysis by a reference laboratory (*see subsection 2.1*).

With regard to length of time-series, the GMP guidance document considers it naïve to expect monitoring of POPs to reveal temporal trends with any confidence within a sampling period of five years unless the changes are very large. More likely would be a period of at least 10–15 years to detect significant changes of moderate size (5%/year) (UNEP 2019). This assessment comprises much longer periods: The first WHO-coordinated exposure study with the aim to determine concentrations of non-dioxin-like PCB (expressed as the sum of 6 indicator PCB [ΣPCB_6]), PCDD and PCDF took place in 1987–1988 (WHO 1989). The second study (1992–1993) not only included the 6 non-dioxin-like indicator PCB, but also dioxin-like PCB (WHO 1996). With inclusion of these two studies, data from a total of 57 countries with repeated participation between 1987 and 2019 are available for assessment of temporal trends for PCB, PCDD and PCDF. In this article, this comprehensive database is evaluated to derive time trends for non-dioxin-like PCB and the toxic equivalents (TEQ) of PCDD and PCDF for a period of 32 years starting with the period 1987–1988 and for the total TEQ comprising PCDD, PCDF and dioxin-like PCB for a period of 27 years starting with 1992–1993.

2 General Aspects

2.1 Minimization of Sources of Variation

Numerous factors might affect the measured concentration of POPs in human milk samples. Sampling design is considered to be the most important factor. For most POPs, the precision of chemical analysis is generally believed to constitute only a minor part of the total variance in monitoring time-series of environmental data where sample variation is expected to be large, much larger compared to laboratory precision. This is especially true when the same accredited laboratory is used throughout the whole series as in this case. However, if, from year to year, different laboratories carry out the analysis, it could seriously decrease or disable the possibility to evaluate time-series of, for example, POPs. The same is true if the same laboratory changes its methodology (UNEP 2007, 2015, 2019).

To minimize possible sources of variation for time trend analysis of POPs, including PCB, PCDD and PCDF covered by this article, the concept of the WHO/UNEP-coordinated exposure studies has the following basic elements:

1. For minimization of possible sources of variation from the sampling design:
   (a) collection of a large number of individual samples from mothers based on the standardized WHO/UNEP protocol;
   (b) from equal amounts of the individual samples, preparation of pooled samples that are considered to represent the average levels of POPs in human milk for a country or subgroup/region of that country at the time of sampling *(for more information on the sampling design, see subsection 2.2)*;
2. For minimization of the variation from chemical analysis: determination of chlorinated and brominated POPs in the pooled samples by the WHO/UNEP Reference Laboratory for the 2000–2019 studies applying long-term analytical quality control *(see subsection 2.3)*.

2.2 Samples and UN Regional Groups

An overview of the scope, protocols for collection of samples and participation of countries is given in Part I (Malisch et al. 2023a). In brief, in all rounds, the design was based on collection of a number of individual samples and preparation of pooled samples following a standardized protocol that was supervised by national coordinators. Equal aliquots of individual samples were combined to give composite samples, which are considered representative of the average levels of the analytes of interest in human milk for a certain country or subpopulation of a country at the time of sampling.

During the five studies conducted from 2000 to 2019, a total of 232 pooled samples were submitted for analysis by 82 countries. 50 countries participated in more than one of these studies. The detailed data for all 232 pooled samples is contained at the POPs Global Monitoring Plan (GMP) Data Warehouse and can be publicly viewed and retrieved (GMP DWH 2020).

Furthermore, results of countries that participated in the first (1987–1988) and second rounds of WHO-coordinated exposure studies on concentrations of PCB and PCDD/PCDF in human milk (WHO 1989; WHO 1996) were included resulting in a total of 57 countries with repeated participation for these compounds.

In accordance with the implementation of the GMP, parties report through one of the five United Nations Regional Groups. Therefore, countries are classified according to one of these five UN Regional Groups (geopolitical groups), namely the African Group, the Asia-Pacific Group, the Group of Latin American and Caribbean Countries (GRULAC), the Eastern European Group and the Western European and Others Group (WEOG). Note that Australia, New Zealand and the USA (being informally a member) are included as "Others" in the WEOG category (for participating countries and regional distribution, see Malisch et al. 2023a).

2.3 Sum Parameters and Long-term Quality Control

The following sum parameters are used: (1) sum of six Indicator PCB (**ΣPCB_6**) for non-dioxin-like PCB, (2) sum of toxic equivalents (TEQ) of PCDD/PCDF (**WHO-PCDD/PCDF-TEQ**) and (3) total sum of toxic equivalents ("Total TEQ") of mixtures of PCDD/PCDF and dioxin-like PCB (**WHO_{2005}-TEQ**). For calculation, see Malisch et al. 2023b.

All concentrations are reported on a lipid basis.

As an accredited laboratory since 1998, a comprehensive quality control program has been applied by the reference laboratory to assure the long-time reliability of results of human milk samples received for WHO/UNEP-coordinated exposure studies between 2000 and 2019. This included procedural blanks, the use of fortified vegetable oil and numerous quality control samples as an in-house reference material, duplicate analyses, and successful participation in 32 proficiency tests (PTs) covering 81 samples of food of animal origin or human milk. Trueness was estimated from the PT samples in the relevant range for human milk above 1 pg WHO-TEQ/g lipid: The deviation was less than 10% from the assigned values for WHO-PCDD/PCDF-PCB-TEQ and WHO-PCDD/PCDF-TEQ and less than about 15% for WHO-PCB-TEQ for about 90% of the results. For the sum of six indicator PCBs (relevant occurrence range, 1–1000 ng/g lipid), approximately 90% of the results differed by less than 15% from the assigned values. A long-term precision of $<$15% (coefficient of variation of within-laboratory reproducibility) was achieved, based on quality control samples analysed during 2000 and 2019. The analytical methodology used, fulfilled the requirements of the general criteria for the operation of testing laboratories as laid down in EN ISO/IEC 17025:2018, the analytical criteria for PCDD/PCDF and PCB in feed and food specified in EU legislation and the criterion for monitoring information for Parties to the Stockholm Convention (Malisch and Schächtele 2023).

2.4 Methods of Statistical Data Treatment

Each pooled sample (considered to be representative for a certain country or subpopulation of a country at the time of sampling) was identified by country, UNEP region, year, and analyte and its concentration reported on lipid basis. In the first three studies, countries were encouraged to submit at least two pooled samples, whereas in the following rounds, in most cases, only one pooled sample was submitted by a country considered to represent the country in that period. If a country had sent two or more samples in a certain round, the median of these samples in this period has been used for the country in some summarizing figures and tables introducing into a section for a certain analyte. These are identified as "country results" although data result from aggregation of multiple sample results. However, for the time trend analysis, data were not aggregated, and values of all individual samples were used for time trend analysis.

Based on visually assessed statistical distributions of compound-specific data, the decadic log-transformation was applied before the trend analysis to accomplish two goals:

- to bring the data distributions closer to the normal distribution, which yields less biased trend estimates even in case of non-parametric estimators,
- to enable estimation of exponential trends using common methods for the linear trends (a linear trend becomes exponential after the reverse log-transformation).

Rather than (unrealistic) linear trends, exponential trends as commonly observed in cases after stop of production and application of a chemical were expected considering the first order kinetics of the decrease of the compounds concentration in the population (Sharma et al. 2021). This approach is in line with various elimination studies deriving half-lives for PCB and PCDD/PCDF (EU Scientific Committee on Food 2000; Canady et al. 2002; EFSA 2018).

The GMP guidance document recommends to apply simple linear regression or the Theil-Sen estimator for power analysis of statistical trends (UNEP 2019). Therefore, the non-parametric linear **Theil-Sen trend estimator** (Sen 1968; Theil 1992) was used on log-transformed data for derivation of the trends $c = A \cdot 10^{-s \cdot t}$, where c denotes the compound concentration, t denotes time, A denotes a concentration coefficient and s denotes the speed of the decrease. R package 'Median-based Linear Models (mblm)' (Komsta 2013) was used for this regression.

Based on the trend characteristics, **decreases** (***decrease rate constants***) per 1 year, 4 years (average lengths of the WHO/UNEP-coordinated studies), 5 years and 10 years were computed: (1) in case of the exponential trends in a form of a percentage decrease per year, (2) in case of the trends with baselines in a form of percentage decrease of the concentration above the baseline. (A 10-year period is used by the GMP guidance document for stipulation of the quantitative objective for the ability of temporal studies to detect a 50% decrease in the levels of POPs [UNEP 2015; UNEP 2019]). Statistical significance of differences between the trends with and without the baseline was estimated to show whether it is advantageous to consider the baselines or not. Since we did not find any significant difference between the trend computed with and without the baseline and all the baselines found in the data were lower than 24 pg/g lipid (as maximum for ΣPCB_6) with most of them under 1 pg/g lipid (mainly TEQ-based results), we considered the background concentrations negligible or zero and all other analyses were done without these baselines.

Trends can be derived if the trend test (significance of the Theil-Sen estimator) is positive on 95% confidence level of significance (i.e. p-values <0.05). As the p-value calculated by the Theil-Sen estimator is never below 0.05 for less than 5 data points and for most countries only less than 5 data points were available, statistically significant trends could be derived only for regions (combining data from countries) and few countries, showing on 95% confidence level whether the trend is not caused by random variance in the data. Then, the R^2 value was used as an indicative measure of a goodness of fit. In addition, for some countries, based on

statistically significant decreases (*decrease rate constants*) and participation also in the decade after 2010, a prognosis of the estimated concentrations in 2025 was derived. However, for many countries, only two or three data points are available. In these cases, the observed changes of the concentrations are statistically not significant and indicate *tendencies.*

Considering the data were in general not normally distributed and the non-parametric method was used for the trend estimation, several trends with high *p*-value and low R^2 value were discussed case by case for conclusions.

Simpson's paradox is a statistical phenomenon in which a trend appears in certain groups of data but disappears or reverses when the groups are combined (Simpson 1951). In the assessment of temporal trends, this paradox can cause improper results induced by use of samples from different countries in different times. To prevent this, two additional analyses were made. First, the annual decrease was computed not only over the whole period but also separately for three decades (before 2000, 2001–2010 and after 2011) whenever data were available. Slopes of trends in these three decades were then compared using z-scores (Fisher test of differences between trends) (Fisher 1915). Second, a method of deriving the regional trend as a median of trends in countries within the region was used ("**median method**"): For each country, one trend was estimated using the Theil-Sen estimator. Median slope and median intercept over all these trends within a UN region were then considered as the resulting regional trend. The z-score test was used to compare this "median trend" with the regional trend derived before using the Theil-Sen trend estimator.

2.5 Background Concentrations Versus High Concentrations after Exposure

For the purpose of these chapters, a (low) background concentration is defined as that portion of the measured human milk levels that is found in the absence of specific sources (e.g. use or emission) and, therefore, is not attributable to a known exposure source within the study area. In contrast, after use or emission of chemicals initially high concentrations of these substances may be found in human milk. However, after a sufficiently long phase-out period the levels of many POPs may decrease considerably and approach background levels. According to the Global Monitoring Plan background levels of POPs are found at locations not influenced by local sources.

Reduction rates should be seen in context with the concentration range (levels above or in the range of background contamination): If high levels are found, sources might be detected, which could be reduced or eliminated and would then result in decreasing trends. However, at low background levels, other factors, e.g., contamination of feed and food by air via long-range transport or from subsequent bioaccumulation, cannot be influenced locally, with the possible consequence of levelling out of the found concentration or some fluctuation of the calculated decreases (*decrease rate constants*).

An additional assessment was done considering a case with background concentration (i.e. not purely an exponential trend). **Non-linear regression using the PORT method** (Fox 1984) implemented in R package stats was used to fit trends of a formula $c = A \cdot 10^{-s \cdot t} + c_0$ where c_0 denotes the background concentration corresponding to the time invariant persistent exposition to the compounds.

PCB are industrial chemicals that were manufactured for decades before their production and use was banned by many countries around 1985. Therefore, background levels of PCB in human milk are the result of implementation of a reduction and control policy for this chemical.

PCDD/PCDF are unintentional by-products formed in (1) a number of chemical processes and, therefore, found as contaminants in certain chemicals; (2) many combustion processes and (3) certain geological processes and are, therefore, present in certain clays. The UNEP toolkit for identification and quantification of releases of dioxins, furans and other unintentional POPs lists a wide range of possible sources (UNEP 2013). Although PCDD/PCDF are regarded as unintentionally produced POPs, their release is mainly caused by intentional human activities (Wang et al. 2016). Therefore, background concentration of PCDD/PCDF is understood here as what is found after implementation of a reduction policy for sources from anthropogenic activities and a sufficiently long phase-out period.

However, the term "background" does not imply per se any level of safety. With respect to potential adverse effects, risk assessments need to consider many factors, including the toxicity of the chemical of interest and the measured concentration range. For human milk, potential adverse effects have to be balanced against its many known positive health aspects for (breast-fed) infants. The risk–benefit evaluation of breastfeeding for dioxin-like compounds is part of the assessment chapters in Part IV.

3 Non-Dioxin-Like Polychlorinated Biphenyls (Indicator PCB, ΣPCB_6)

Time trends for six non-dioxin-like PCB (Indicator PCB, ΣPCB_6) can be derived beginning with the 1987–1988 period. In total, 140 country results (including data from aggregation of multiple pooled samples that were averaged to represent a country in a certain period) for 57 countries with repeated participation in the period 1987–2019 and 119 aggregated data for 50 countries for the period 2000–2019 were used as basis for the first *general* estimation of time trends. In 7 periods between 1987 and 2019, the median of the ΣPCB_6 concentrations decreased considerably from 211 ng/g to 14 ng/g (= 93% decrease). The Stockholm Convention was adopted in 2001 and entered into force in 2004 (UNEP 2001). Therefore, if only the period 2000–2019 is taken into consideration, the median concentrations of ΣPCB_6 went down by 89% from initially 123 ng/g in the period 2000–2003 (comprising the year 2001) and by 71% from initially 66 ng/g in the period 2004–2007 (comprising the year 2004) (Table 1).

Table 1 Ranges of indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data using the median if two or more pooled samples were submitted by a country in a certain period) in 7 periods between 1987 and 2019 for countries with two or more participations; change of the median concentrations calculated as % of round 1 (1987–1988) and round 3 (2000–2003), respectively, as points of reference

			ΣPCB_6 (ng/g lipid)			Change of median concentrations over time	
Round	Period	No of countries	Min	Median	Max	% relative to round 1	% relative to round 3
1	1987–1988	7	62	211	762	100%	
2	1992–1993	14	56	275	801	130%	
3	2000–2003	24	16	123	502	58%	100%
4	2004–2007	11	10	66	376	31%	54%
5	2008–2011	36	4	23	79	11%	18%
6	2012–2015	15	2	36	158	17%	29%
7	2016–2019	33	1	14	109	7%	11%

These summarizing descriptive parameters seem to indicate fluctuations between decreasing and increasing periods, e.g. an increase of the median concentrations from the first to the second and from the fifth to the sixth round. However, these changes are likely due to the result of participation of different countries in different rounds. Therefore, for more precise time trends a country-specific evaluation is necessary.

3.1 European and Non-European Countries

For PCB, as a first step, the differentiation between European and non-European countries is necessary. As the total results for 232 pooled human milk samples of 82 countries (regardless of the number of participations in the WHO/UNEP-coordinated exposure studies) for the period 2000–2019 showed, European countries used to have higher PCB concentrations than non-European countries with the greatest difference in the initial rounds (Malisch et al. 2023b): The highest concentrations of the sum of six Indicator PCB were found in the Eastern European Group (median about 120 ng ΣPCB_6/g lipid, maximum about 1000 ng ΣPCB_6/g lipid), followed by the group of Western European and Other States (median about 75 ng ΣPCB_6/g lipid, maximum 467 ng ΣPCB_6/g lipid). In all other groups, considerably lower ΣPCB_6 levels were found (median approximately between 8 and 22 ng/g, maximum slightly lower than 100 ng/g lipid). The minimum in 24 European countries (14.6 ng ΣPCB_6/g lipid) equated approximately the median (16.4 ng ΣPCB_6/g lipid) in 58 non-European countries (with a minimum of 0.9 ng ΣPCB_6/g lipid). Thus, within the wide range of concentrations varying approximately between 1 ng/g lipid and 1000 ng/g lipid, the lower end between 1 ng/g lipid and 10 ng/g lipid might be seen as background contamination in non-European

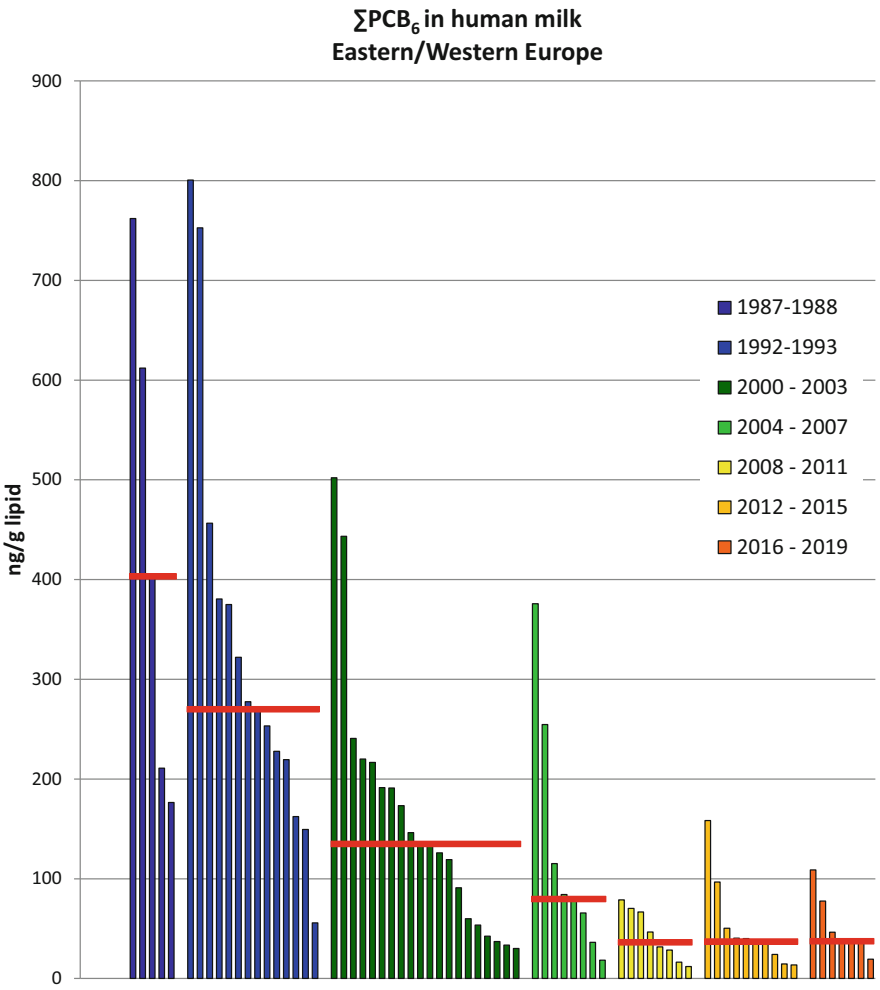

Fig. 1 Overview of Indicator PCB concentrations in human milk in seven periods between 1987 and 2019 with indication of temporal tendencies as median in the respective studies for 25 countries from the Eastern European Group and the Western European and Others Group with two or more participations (ng ΣPCB_6/g lipid; aggregated data; red line for the median)

countries, whereas the upper part of the frequency distribution of ΣPCB_6 concentrations is an indication for former use.

This wide range is also illustrated by the summarizing overviews (using aggregated data) of the results for ΣPCB_6 of 25 countries from the Eastern European and Western European and Others groups (Fig. 1) in comparison with 32 countries from the African, Asia-Pacific and Latin American and the Caribbean regional groups (Fig. 2), all of them with repeated participation between 1987 and 2019: In European countries, ΣPCB_6 were at the end of the 1980s and early 1990s in

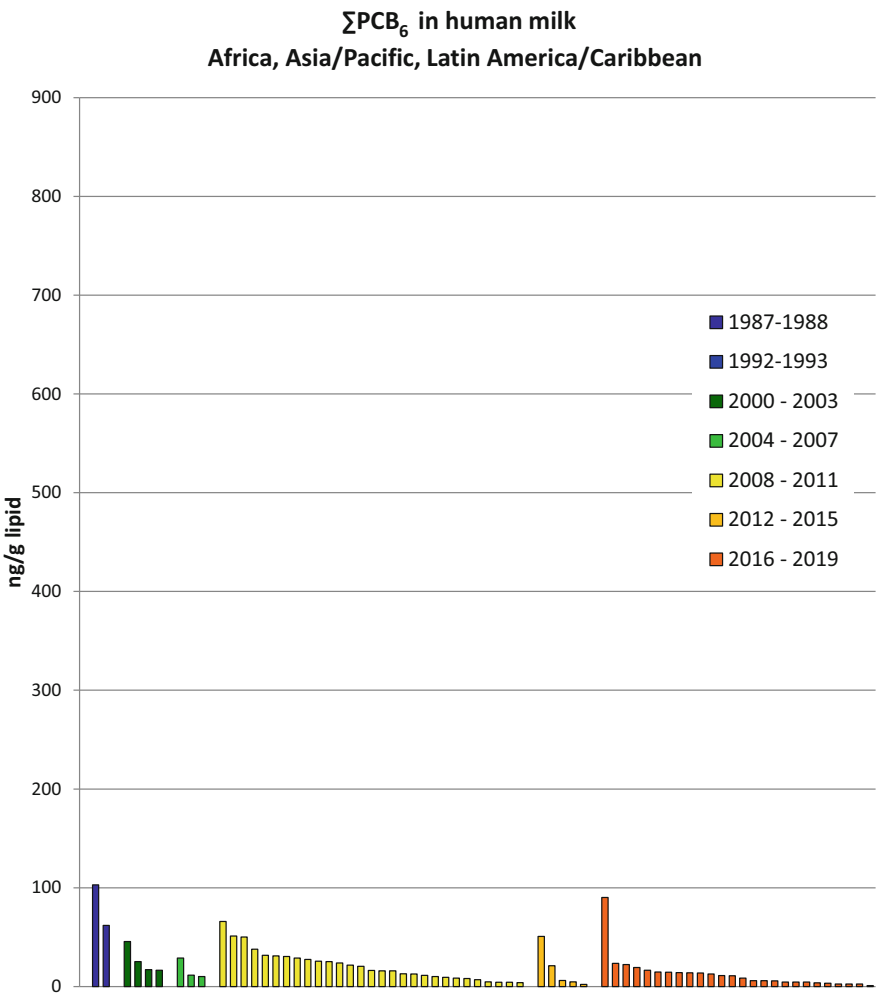

Fig. 2 Overview of Indicator PCB concentrations in human milk in seven periods between 1987 and 2019 for 32 countries from the African, Asia-Pacific and Latin America and the Caribbean regional groups with two or more participations (ng ΣPCB_6/g lipid; aggregated data). *(This figure is normalized to 900 ng/g lipid as maximum value as in* Fig. 1 *allowing a direct visual comparison of the ΣPCB_6 concentrations among the different collection periods in European and non-European countries)*

a range up to 800 ng/g lipid and decreased to 2019 to concentrations mostly below 100 ng/g lipid. These levels are approximately the maximum concentrations found in other UN Regional Groups over the whole period 1987–2019. Note that these two figures are normalized to 900 ng/g lipid as maximum value allowing a direct visual comparison of the ΣPCB_6 concentrations among the different collection periods as an indication of time trends.

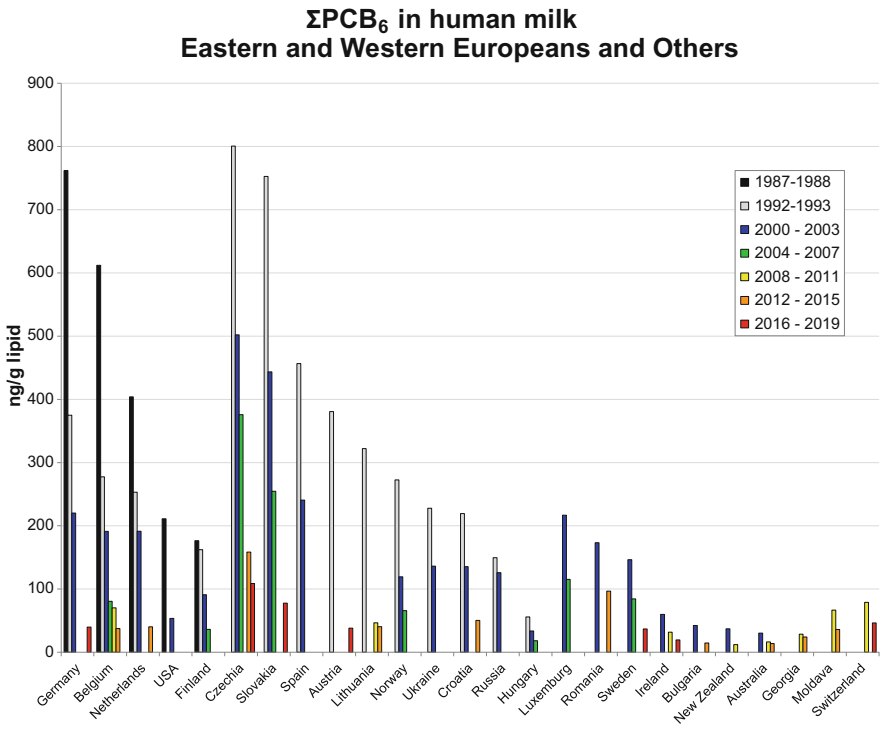

Fig. 3 Overview of the development of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data) over time between 1987 and 2019 for 25 countries of the Eastern European and Western European and Others regional groups that participated two or more times in the WHO/UNEP-coordinated surveys

Figure 3 illustrates the time trends of ΣPCB_6 concentrations (aggregated data) between 1987 and 2019 in the 25 individual countries of the Eastern European and Western European and Others groups. Significant reductions were already achieved in the 1990s. After detection of PCB in environmental samples in 1966 (Jensen 1966; Jensen 1972), PCB were detected for the first time in human tissue, including human milk, in 1970 (Acker and Schulte 1970a; Acker and Schulte 1970b; Schulte 1971; Schulte and Acker 1974a; Schulte and Acker 1974b). As a result, during 1971/2 the PCB producers of the Western world (at that time only 4 remained) introduced voluntary restrictions on use. In 1973 the OECD recommended the use of PCB only in closed systems, which were adopted into national regulations, e.g., by Germany in 1978. Therefore, in Germany, a decrease of ΣPCB_6 concentrations in human milk by 95% was achieved between the end of the 1980s and culminated in low background levels in 2019. Similarly successful were Belgium, the Netherlands, the Czech Republic (Czechia), the Slovak Republic (Slovakia), Austria and Lithuania with reductions in the range of 85% to 95% between the end of the 1980s/early 1990s and the period between 2012 and 2019. Also, all other countries had substantially

reduced ΣPCB_6 concentrations in human milk, but mostly in a shorter period, at least in cases for which data are available.

Also, in most countries from the regions of Africa, the Asia-Pacific and Latin American and Caribbean, a decrease between different periods was observed, but these decreases began at considerably lower concentrations. These results are discussed in more detail in the following sections.

3.2 Global Level and Comparison Between UN Regional Groups

For time trend analysis of ΣPCB_6 between 1987 and 2019, results of 247 pooled samples were available. In two Asian and many European countries PCB were monitored over three decades, whereas the studies in African, Latin American and Caribbean countries comprise two decades with most of these countries participating for the first time in the 2008–2011 period. Basic results of the exponential trends comprise the overall decrease per 1 year and 10 years (Table 2). For the country-specific results in the following subsections, also the decrease per 5 years is shown, which is about 20% higher than the decrease per 4 years representing the average lengths of WHO/UNEP-coordinated exposure studies.

In nearly all UN regions and at a global level, a 50% decrease within a 10-year period (corresponding to an annual decrease of 7%) was achieved for the levels of ΣPCB_6. The Latin American and Caribbean countries had lower ΣPCB_6 concentrations in comparable periods, obviously resulting in lower decrease rates. This is an indication that the decrease might be faster in regions with higher concentration, compared to a slower decrease in less polluted regions. All trends were statistically significant (p-value <0.001).

Table 2 Overall decrease (%) of indicator PCB concentrations in human milk in the 5 UN Regional Groups and worldwide (computed using all individual samples, see subsection 2.4)

UN Regional Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall[a]
		Theil-Sen method	median method	Theil-Sen method	median method	
Africa	13	6.6	8.0	49.5	56.7	<0.001
Asia-Pacific	10	9.9	6.9	64.8	51.2	<0.001
Latin America and Caribbean	9	4.1	5.8	34.0	45.1	<0.001
Eastern Europe	11	7.3	7.9	53.3	55.8	<0.001
Western Europe and Others	14	9.4	9.2	62.8	61.9	<0.001
Global	57	11.5	7.3	70.5	53.3	<0.001
Median of UN Regional Groups			*7.9*		*55.8*	

[a]For Theil-Sen method

Statistical differences between the Theil-Sen method and the median method to derive time trends in different UNEP Regional Groups were insignificant on 95% confidence level, which shows that the Simpson paradox caused by different sampling periods is weak in these cases. On a global level calculated as the median of all samples, the annual decrease was 11.5% (i.e. half-life of 5.7 years) computed by the Theil-Sen method, compared with an annual decrease of 7.3% in the case of the median method.

The exponential trends of indicator PCB derived by the Theil-Sen method in the five UN Regional Groups and worldwide are illustrated in Fig. 4a–f.

3.3 African Group

Figures 5 (for aggregated data) and 6 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of ΣPCB_6 concentrations in 13 countries from Africa with repeated participation between 2000 and 2019. In Egypt, a reduction of approximately 85% was observed from the median of 9 pooled samples of various regions submitted in the 2000–2003 period to the one pooled sample submitted in the 2016–2019 period, which is considered to represent the country at that time. Most countries participated for the first time in the 2008–2011 period; in these countries, ΣPCB_6 concentrations fell on average by about 50% until the period 2016 to 2019 (range between decrease by 71% and increase by 37%). Ethiopia also had the lowest levels of all countries in the 2000–2019 studies for this parameter: 2.15 ng ΣPCB_6/g lipid in 2012 with a downward trend even of this low background level to 0.90 ng/g lipid in 2019. In Côte d'Ivoire, the ΣPCB_6 concentrations remained unchanged over the observed shorter period (between 2008–2011 and 2012–2015). An increase was observed in Senegal.

The overall decreases per 1 year, 5 years and 10 years are given in Table 3. In nearly all African countries concentrations tend to decrease. A decrease around 50%–60% was observed, even with low background levels (median for 11 countries with downward trends for the decrease per 10 years: 60%; range 43%–88%). ΣPCB_6 concentrations remained quite constant in Côte d'Ivoire and increased by 42% in Senegal within a 10-year period. A statistically significant decrease of 88% over 10 years was found in Egypt ($p < 0.001$). In the other countries, the limited number of samples did not allow to determine a statistically significant decrease ($p \sim 1.000$) (for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). However, for all African countries on average, the decrease over 10 years of 50% (calculated by the Theil-Sen method and use of all individual samples) was statistically significant ($p < 0.001$) (see Table 2 in subsection 3.2).

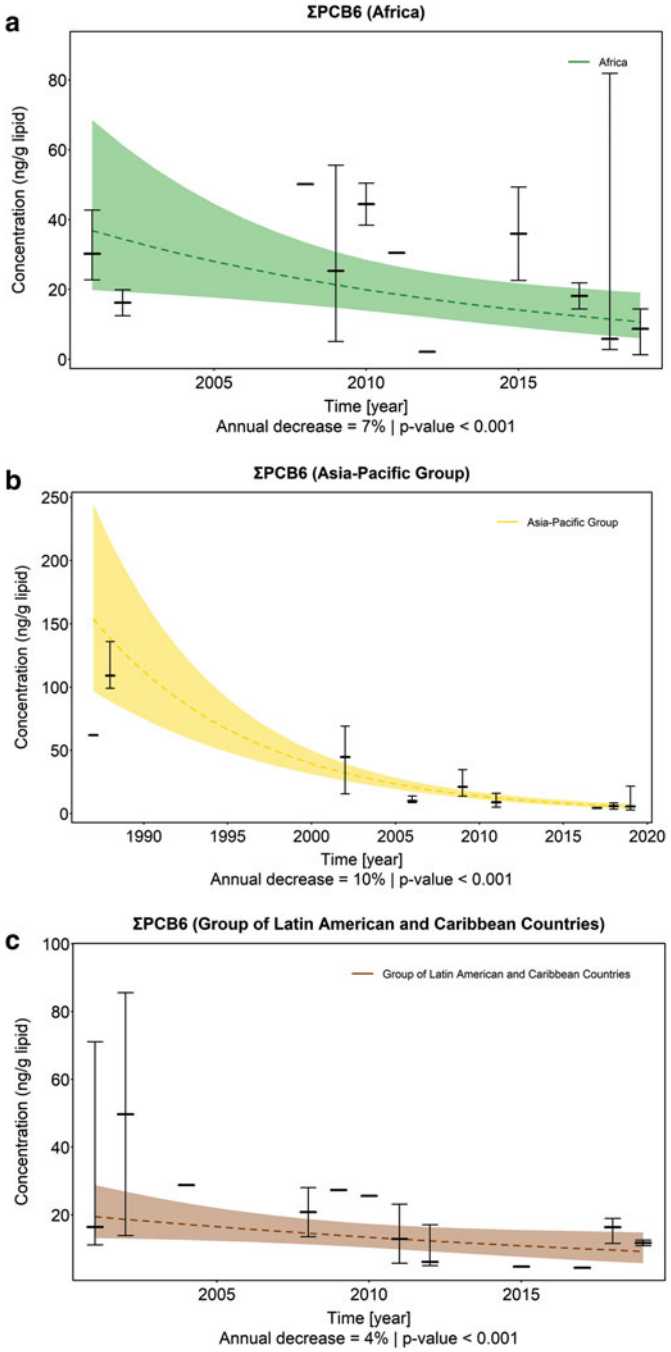

Fig. 4 (**a**–**f**) Theil-Sen exponential trends of indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid) in the five UN Regional Groups and worldwide. The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, whiskers show ranges between fifth and 95th percentiles

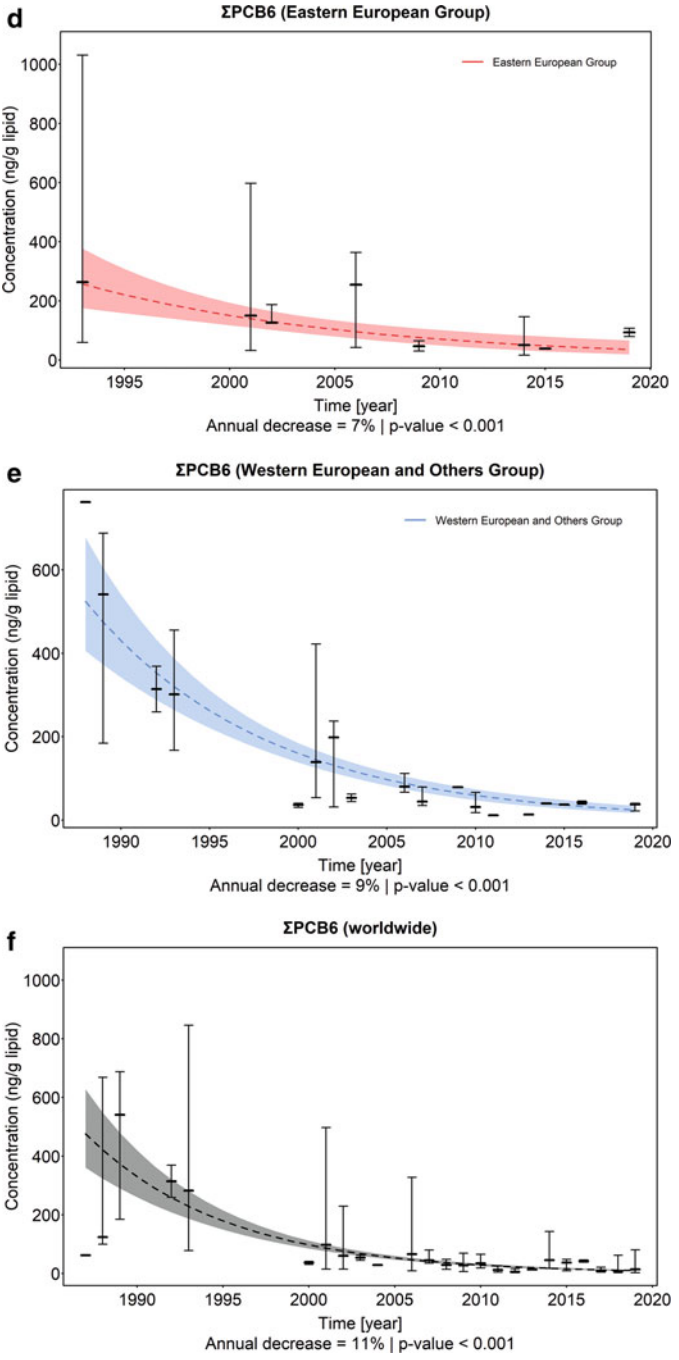

Fig. 4 (continued)

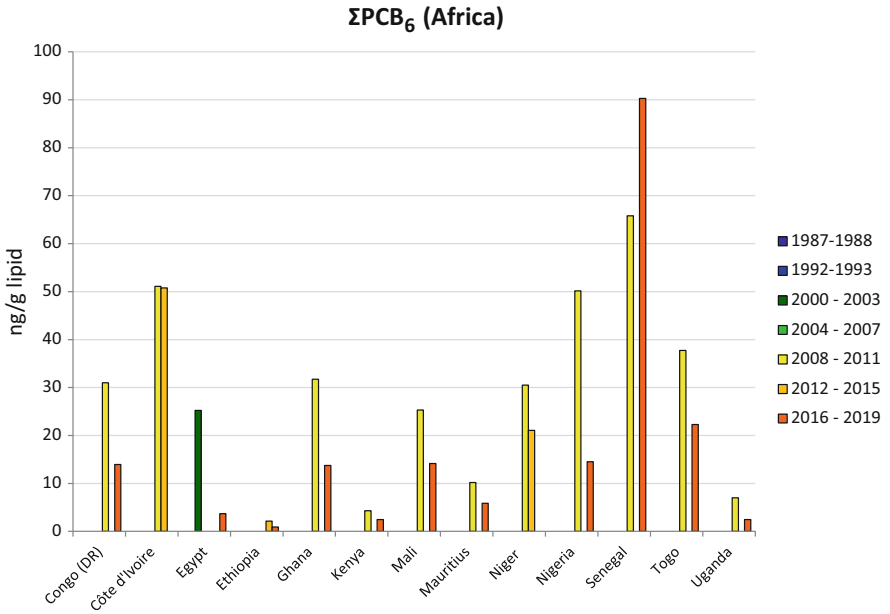

Fig. 5 Overview of the development of indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data) over time for African countries with repeated participation between 2000 and 2019

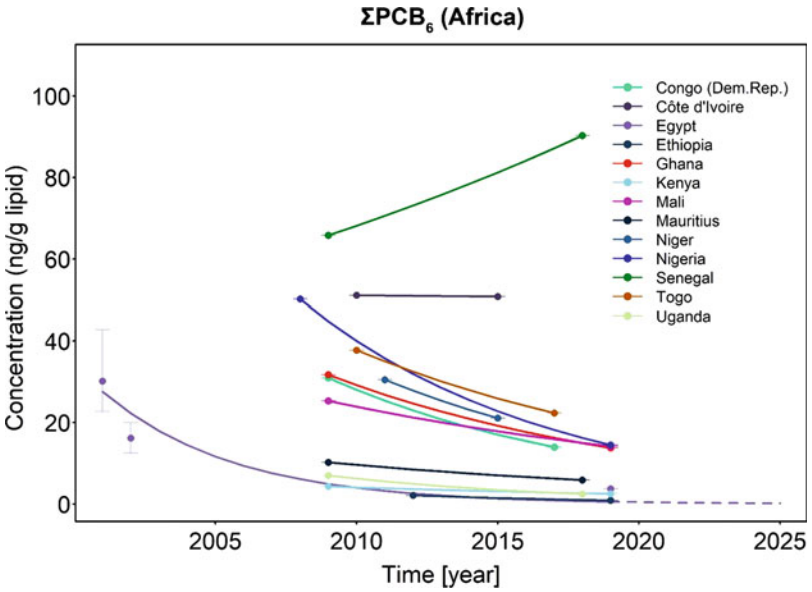

Fig. 6 Temporal tendencies of indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid) for African countries with repeated participation between 2000 and 2019 using the Theil-Sen method

Table 3 Overall decrease (%) of Indicator PCB concentrations in human milk per 1 year, 5 years and 10 years in African countries (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Congo (DR)	9.5	39.2	63.0	1.000
Côte d'Ivoire	0.1	0.6	1.2	1.000
Egypt	19.4	65.9	88.4	<0.001
Ethiopia	11.7	46.4	71.2	1.000
Ghana	8.0	34.2	56.7	1.000
Kenya	5.4	24.3	42.7	1.000
Mali	5.6	25.2	44.1	1.000
Mauritius	6.0	26.5	45.9	1.000
Niger	8.8	37.0	60.3	1.000
Nigeria	10.7	43.1	67.6	1.000
Senegal	−3.6	−19.2	−42.1	1.000
Togo	7.2	31.3	52.8	1.000
Uganda	11.0	44.0	68.7	1.000
Median	8.0	34.2	56.7	

3.4 Asia-Pacific Group

Figures 7 (for aggregated data) and 8 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of ΣPCB_6 concentrations in 10 countries from the Asia-Pacific Group with repeated participation between 1987 and 2019. As in European countries, Vietnam and Thailand also revealed a decrease of 85% to 95% between the end of the 1980s and 2016–2019, however, at considerably lower concentrations in both periods. In Fiji, a reduction of approximately 75% was observed from 2000–2003 to 2008–2011, which then remained stable until 2016–2019. In Hong Kong SAR of China, ΣPCB_6 concentrations fell approximately 50% from 2000–2003 to 2008–2011. Most countries of the Pacific subregion participated for the first time in the period 2008–2011; Kiribati in 2004–2007. In nearly all Pacific Island countries, a decrease was observed in the following years even with the low initial background levels.

The overall decreases per 1 year, 5 years and 10 years are given in Table 4. A decrease in the levels of ΣPCB_6 within a 10-year period around 50% was achieved by most countries even with the low range of background contamination (median for 9 countries with downward trends for decreasing rates per 10 years: 55%; range 35%–64%). Statistically significant was the decrease of 64% over 10 years in Hong Kong SAR of China ($p < 0.001$) and of 55% in Fiji ($p = 0.008$). In the other countries, the limited number of samples did not allow determination of a statistically significant decrease ($p = 0.250$ and 1.000, respectively) (for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating

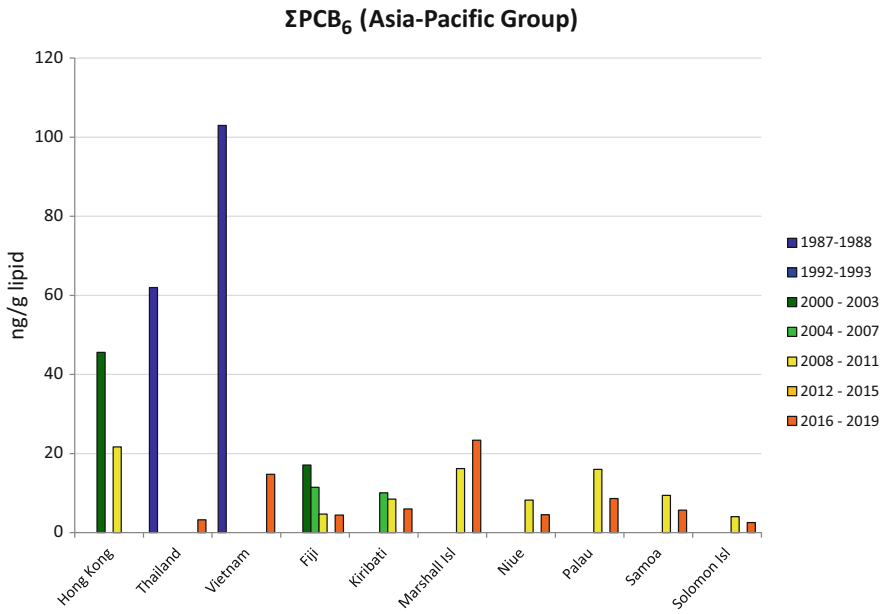

Fig. 7 Overview of the development of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data) over time for countries of the Asia-Pacific Group with repeated participation between 1987 and 2019

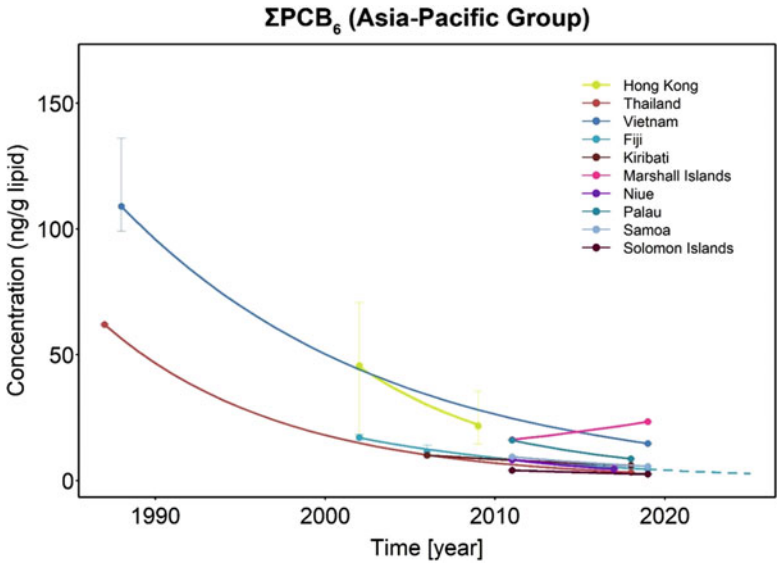

Fig. 8 Temporal tendencies of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid) for countries of the Asia-Pacific Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 4 Overall decrease (%) of indicator PCB concentrations in human milk per 1 year, 5 years and 10 years in countries of the Asia-Pacific Group and for one country estimated concentrations (ng ΣPCB_6/g lipid) in 2025 (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Fiji	7.6	32.6	54.6	2.4	0.008
Hong Kong	9.6	39.7	63.7		<0.001
Kiribati	4.2	19.4	35.0		0.250
Marshall Isl.	−4.7	−25.7	−58.0		1.000
Niue	9.5	39.2	63.0		1.000
Palau	8.5	35.7	58.7		1.000
Samoa	6.1	27.1	46.8		1.000
Solomon Isl.	5.6	25.1	43.9		1.000
Thailand	9.1	37.8	61.4		1.000
Vietnam	6.2	27.6	47.5		0.250
Median	6.9	30.1	51.2		

tendencies, see subsection 2.4); however, for the Asia-Pacific Group on average, the decrease over 10 years of 65% (calculated by the Theil-Sen method and use of all individual pooled samples) was statistically significant ($p < 0.001$) (see Table 2 in subsection 3.2). For Fiji with a statistically significant decrease and participation also in the 2016–2019 period, also a prognosis of the estimated concentrations in 2025 was derived.

3.5 Group of Latin American and Caribbean Countries (GRULAC)

Figures 9 (for aggregated data) and 10 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of ΣPCB_6 concentrations in 9 Latin American and Caribbean countries with repeated participation between 2000 and 2019. In Brazil a reduction of 62% was found from the median of 10 pooled samples of the 2000–2003 period to the median of 3 pooled samples of the 2012–2015 period. Most other countries participated for the first time in the period 2008–2011 with Haiti in 2004–2007. In nearly all countries, a decrease was observed in the following years. In Mexico, the background levels remained unchanged.

The overall decreases per 1 year, 5 years and 10 years are given in Table 5. A decrease in the levels of ΣPCB_6 around 50% within a 10-year period was achieved by most countries (median for 8 countries with decreasing rates per 10 years: 51%; range 30%–81%). ΣPCB_6 concentrations remained stable at the background levels found in Mexico. Statistically significant was the decrease of 56% over 10 years in Brazil ($p = 0.022$). In the other countries, the limited number of samples did not

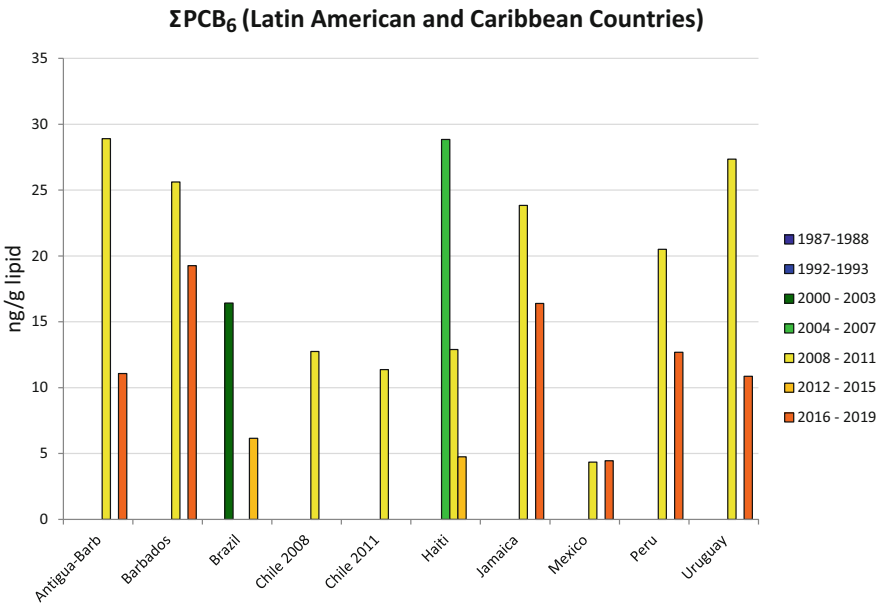

Fig. 9 Overview of the development of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data) over time for countries of the Group of Latin America and the Caribbean with repeated participation between 2000 and 2019

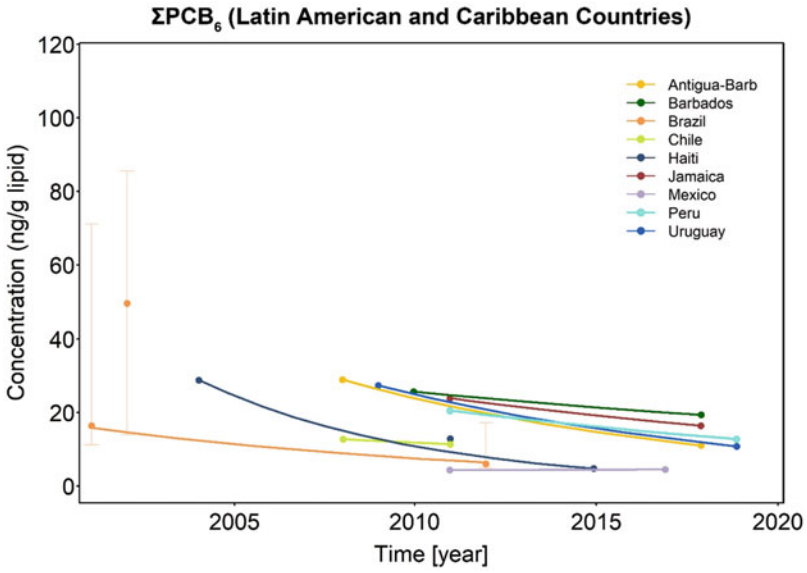

Fig. 10 Temporal tendencies of indicator PCB concentrations in human milk (ng ΣPCB6/g lipid) for countries of the Group of Latin America and the Caribbean with repeated participation between 2000 and 2019 using the Theil-Sen method

Table 5 Overall decrease (%) of indicator PCB concentrations in human milk per 1 year, 5 years and 10 years in countries of the Group Latin America and the Caribbean (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Antigua-Barb	9.1	38.1	61.7	1.000
Barbados	3.5	16.3	30.0	1.000
Brazil	7.9	33.8	56.2	0.022
Chile	3.7	17.3	31.6	1.000
Haiti	15.1	56.0	80.6	0.250
Jamaica	5.2	23.4	41.4	1.000
Mexico	−0.4	−2.1	−4.2	1.000
Peru	5.8	25.9	45.1	1.000
Uruguay	8.8	37.0	60.3	1.000
Median	5.8	25.9	45.1	

allow to determine a statistically significant decrease (for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). However, for all participating Latin American and Caribbean countries on average, the decrease over 10 years of 34% (calculated by the Theil-Sen method and use of all individual samples) was statistically significant ($p < 0.001$) (see Table 2 in subsection 3.2).

3.6 Eastern European Group

Figure 11 (for aggregated data) and Fig. 12 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of ΣPCB_6 concentrations in 11 countries of the Eastern European Group with repeated participation between 1987 and 2019. A continuous decrease is observed in all countries over all periods from the high concentrations found in the 1992–1993 period and decreasing considerably during the following rounds and later levelling out. The highest concentrations were found in 1992–1993 in Czechia (median of three pooled samples: 800 ng ΣPCB_6/g lipid) and in Slovakia (median of three pooled samples: 753 ng ΣPCB_6/g lipid). These decreased until 2019 in Czechia by 86% and in Slovakia by 90%. In 6 countries participating in the 1992–1993 study, the ΣPCB_6 concentrations decreased by approximately 40% until the 2000–2003 period (range 16%–41%, based on the median of submitted pooled samples).

The overall decreases per 1 year, 5 years and 10 years are given in Table 6. A decrease in the levels of ΣPCB_6 within a 10-year period of approximately 50% to 60% was achieved by most countries. ΣPCB_6 concentrations slightly decreased in Georgia between the periods 2008–2011 and 2012–2015. Whereas in Russia the median of two pooled samples collected in the 1987–1988 period decreased to the median of 7 pooled samples submitted in the 2000–2003 period, the Theil-Sen

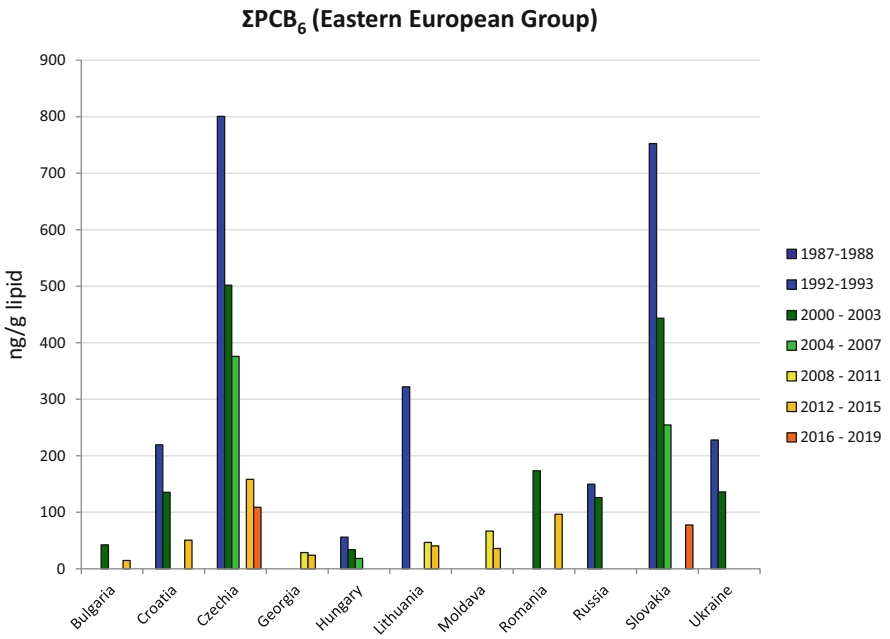

Fig. 11 Overview of the development of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data) over time for countries of the Eastern European Group with repeated participation between 1987 and 2019

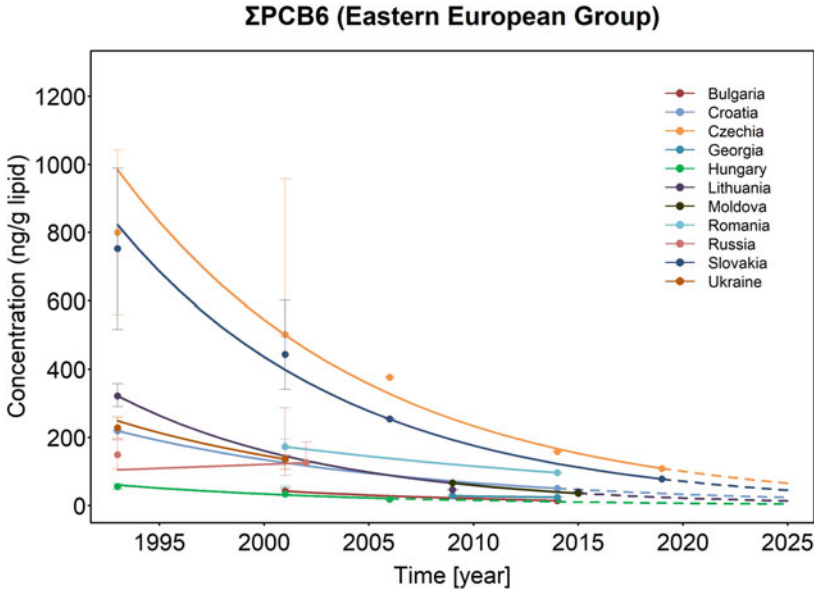

Fig. 12 Temporal tendencies (with many statistically significant time trends) of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid) for countries of the Eastern European Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 6 Overall decrease (%) of indicator PCB concentrations in human milk per 1 year, 5 years and 10 years in countries of the Eastern European Group and for four countries, estimated concentrations (ng ΣPCB_6/g lipid) in 2025 (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Bulgaria	7.9	33.6	55.8		0.250
Croatia	6.8	29.5	50.3	23	0.008
Czechia	8.1	34.5	57.1	65	< 0.001
Georgia	3.3	15.6	28.7		1.000
Hungary	7.9	33.6	55.9		0.003
Lithuania	9.5	39.2	63.0	13	0.016
Moldova	9.8	40.2	64.3		1.000
Romania	4.4	20.1	36.2		0.250
Russia	−2.1	−10.7	−22.6		0.842
Slovakia	8.7	36.4	59.6	45	< 0.001
Ukraine	7.2	31.4	52.9		0.031
Median	7.9	33.6	55.8		

method using all individual pooled samples identified a statistically insignificant increase as a result of the frequency distribution of the 7 samples of the 2000–2003 period in comparison with the 1987–1988 period. Statistically significant ($p < 0.05$) were the decreasing rates between 50% and 63% over 10 years in 6 other countries. For all countries of the Eastern European Group on average, the decrease over 10 years of 53% (calculated by the Theil-Sen method and use of all individual samples) was statistically significant ($p < 0.001$) (see Table 2 in subsection 3.2). Based on a statistically significant decrease and participation also in the decade after 2010, a prognosis of the estimated concentrations in 2025 was also derived for 4 countries.

3.7 Western European and Others Group (WEOG)

Figures 13 (for aggregated data) and 14 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of ΣPCB_6 concentrations in 14 countries of the Western European and Others Group with repeated participation between 1987 and 2019. A continuous decrease is observed in all countries over all periods, with high concentrations found in the 1987–1988 period decreasing over the following rounds and later levelling out. In Germany, which covers the whole period between 1987 and 2019, a decrease of ΣPCB_6 concentrations in human milk by 95% was achieved. Belgium and the Netherlands were similarly successful with reductions in the range of 90% to 94% between the end of the 1980s and the 2012–2015 period.

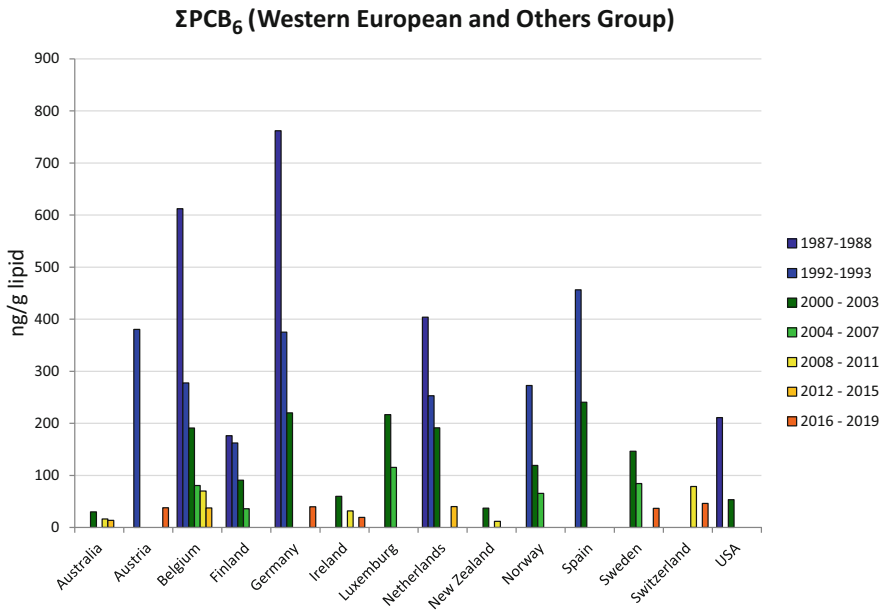

Fig. 13 Overview of the development of Indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid; aggregated data) over time for countries of the Western European and Others Group with repeated participation between 1987 and 2019

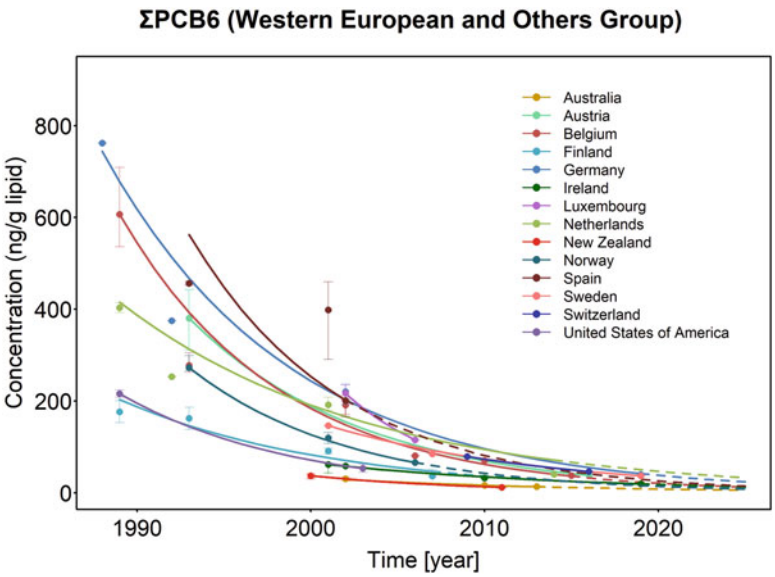

Fig. 14 Temporal tendencies (mostly as statistically significant time trends) of indicator PCB concentrations in human milk (ng ΣPCB_6/g lipid) for countries of the Western European and Others Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 7 Overall decrease (%) of Indicator PCB concentrations in human milk per 1 year, 5 years and 10 years in countries of the Western European and Others Group and for five countries estimated concentrations (ng ΣPCB_6/g lipid) in 2025 (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Australia	6.8	29.8	50.8	5.7	0.008
Austria	9.5	39.4	63.3		0.250
Belgium	10.3	41.9	66.3	12	<0.001
Finland	7.8	33.5	55.8		<0.001
Germany	8.9	37.2	60.5	23	<0.001
Ireland	6.2	27.5	47.4	13	0.034
Luxembourg	14.5	54.3	79.1		0.500
Netherlands	6.8	29.7	50.6	33	<0.001
New Zealand	9.7	40.1	64.1		0.250
Norway	10.4	42.1	66.5		0.001
Spain	10.8	43.4	68.0		<0.001
Sweden	7.4	31.8	53.5		0.250
Switzerland	7.3	31.6	53.2		1.000
USA	9.6	39.6	63.5		0.125
Median	9.2	38.3	61.9		

The median of the decreases for all countries per 10 years was 62% (range 47%–79%). The range between 47% and 68% was statistically significant ($p < 0.05$) in 8 countries (Table 7). For all WEOG countries on average, the decrease over 10 years of 63% (calculated by the Theil-Sen method and use of all individual samples) was statistically significant ($p < 0.001$) (see Table 2 in subsection 3.2). Based on a statistically significant decrease with participation also after 2010, a prognosis of the estimated concentrations in 2025 was derived for 5 countries.

4 Toxic Equivalents of PCDD and PCDF (WHO-PCDD/PCDF-TEQ)

Throughout this publication, all TEQ-related evaluations are based on the WHO-TEFs as proposed in 2005 (Van den Berg et al. 2006). The term "WHO-PCDD/PCDF-TEQ" is used for TEQ of PCDD/PCDF. Results are given as upper bound concentrations and expressed on a lipid basis (Malisch et al. 2023b).

Time trends for WHO-PCDD/PCDF-TEQ can be derived beginning with the 1987–1988 period. For a first general estimation of time trends, 146 aggregated values from data for 57 countries with repeated participation in the period 1987–2019 and 119 aggregated data for 50 countries for the period 2000–2019 were used as the basis. In 7 periods between 1987 and 2019, the median of the concentrations decreased considerably from 16.9 pg/g WHO-PCDD/PCDF-TEQ to

Table 8 Ranges of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid) in 7 periods between 1987 and 2019 for countries with two or more participations and decrease of the median concentrations calculated as % of round 1 (1987–1988) and round 3 (2000–2003), respectively, as point of reference (aggregated data using the median if two or more pooled samples were submitted by a country in a certain period)

			WHO-PCDD/F-TEQ (2005/UB) (pg/g lipid)			Decrease of median over time	
Round	Period	No of countries/ participations	Min	Median	Max	% relative to round 1	% relative to round 3
1	1987–1988	13	5.38	16.9	42.2	100	–
2	1992–1993	14	8.26	11.9	26.0	70	–
3	2000–2003	24	3.08	7.34	18.0	43	100
4	2004–2007	11	2.94	4.83	8.93	29	66
5	2008–2011	36	1.31	3.86	12.1	23	53
6	2012–2015	15	1.01	3.82	8.61	23	52
7	2016–2019	33	1.02	2.68	9.97	16	37
	1987–2019	146	1.01	4.60	42.2		
	2000–2019	119	1.01	3.95	18.0		

2.68 pg/g (= 84% decrease). The Stockholm Convention was adopted in 2001 and entered into force in 2004. Therefore, if only the period 2000–2019 is taken into consideration, the median concentrations went down by 63% from initially 7.34 pg WHO-PCDD/PCDF-TEQ/g in the period 2000–2003. Other than observed for non-dioxin-like-PCB, the WHO-PCDD/PCDF-TEQ concentrations decreased continuously without fluctuations between periods (Table 8).

Figure 15 illustrates the overall picture for temporal trends of concentrations of WHO-PCDD/PCDF-TEQ for 57 countries (using the above-mentioned aggregated data) participating repeatedly in the seven periods between 1987 and 2019.

For 17 countries of the Eastern European and Western European and Others groups and two countries from Asia with repeated participation in the 7 periods between 1987 and 2019, data for WHO-PCDD/PCDF-TEQ are available starting with the first (1987–1988) and second (1992–1993) round of WHO-coordinated exposure studies. Figure 16 illustrates the country-specific results (using aggregated data). A great falling trend of PCDD/PCDF levels in human milk was found with largest declines in countries with the highest initial levels: In the period 1987–1988, the Netherlands, Belgium and Germany had the highest WHO-PCDD/PCDF-TEQ concentrations (ranging 32–42 pg/g as median of two pooled samples from the Netherlands, of three pooled samples from Belgium and of ten pooled samples from Germany). These countries were highly industrialized and made serious efforts in the 1990s to detect and eliminate PCDD/PCDF sources. For example, at the 1990 symposium on dioxins in Germany, new health-based guidance values on proposed acceptable daily intake were derived. To meet these values over time, dioxin sources needed to be detected and eliminated. A major contributor, municipal waste incineration, was identified and a maximum limit of 0.1 ng TEQ/m^3 air was fixed. In

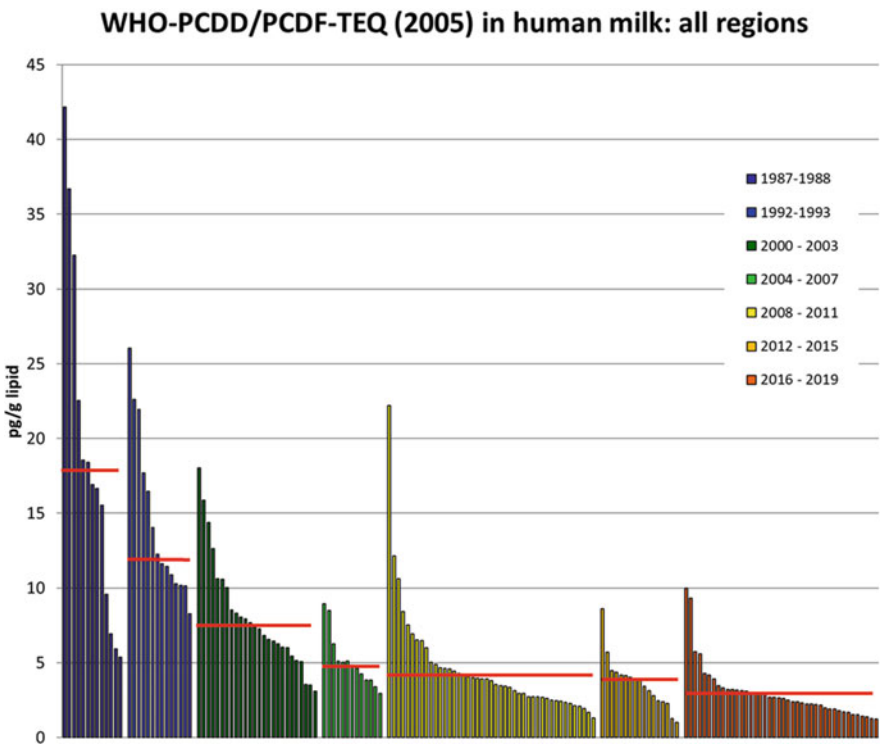

Fig. 15 Overview of WHO-PCDD/PCDF-TEQ concentrations in human milk in seven periods between 1987 and 2019 with indication of temporal tendencies as median in the respective studies for 57 countries with two or more participations (pg WHO-PCDD/PCDF-TEQ/g lipid; red line for the median in a certain round; aggregated data using the median if two or more samples were submitted by a country in a certain period)

addition, regulations on hazardous materials were expanded (Umweltbundesamt und Bundesgesundheitsamt 1990). These efforts were very successful so that annual emissions of PCDD/PCDF in Germany dropped 95% from about 750 g I-TEQ in 1990 to about 70 g I-TEQ in 2004 and stabilized around this level until the end of the observation period in 2011 (Umweltbundesamt 2014). PCDD/PCDF were also released from production of organochlorine chemicals (e.g. PCB, PCP, 2,4,5-T, 2,4-D, chlornitrofen, chloranil) between the 1950s and 1980s, before PCDD/PCDF concentrations in these products as a result of the Seveso accident and regulatory measures were limited (Umweltbundesamt und Bundesgesundheitsamt 1990; Weber et al. 2008).

Over time, similar measures were introduced in other countries and at the EU level. As a result, a substantial decline of about 90% was observed for WHO-PCDD/PCDF-TEQ concentrations in human milk to 4.48 pg/g found in the Netherlands in 2014, 4.03 pg/g found in Belgium in 2015 and 2.90 pg/g found in Germany in 2019. Belgium is the country with the highest number of participations ($n = 6$); its results

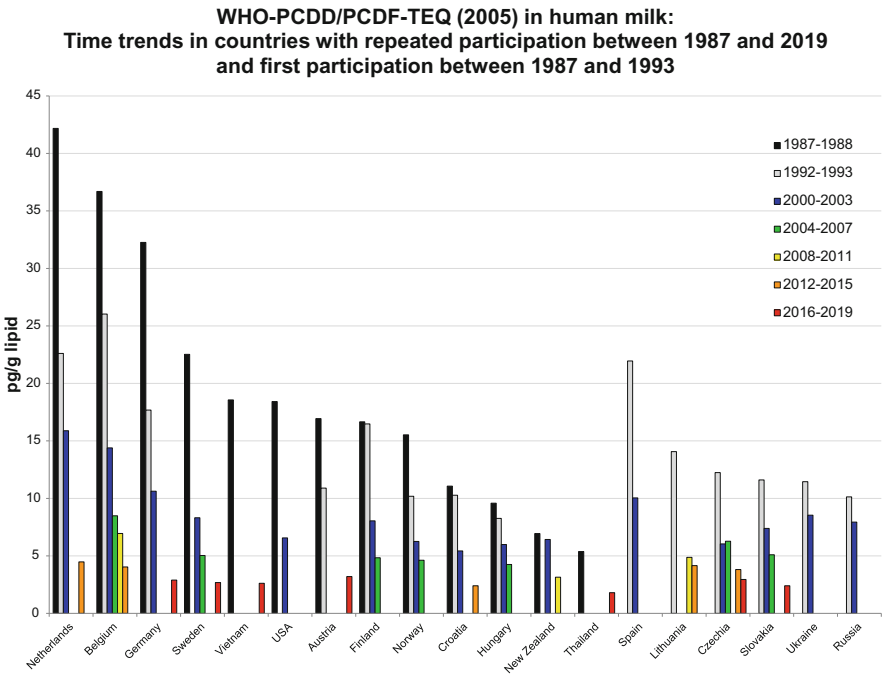

Fig. 16 Overview of the development of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries with repeated participation in WHO/UNEP-coordinated exposure studies between 1987 and 2019 and first participation in the first or second round

show that the most substantial decrease occurred between 1987–1988 (36.7 pg WHO-PCDD/PCDF-TEQ/g) and 2004–2007 (8.49 pg WHO-PCDD/PCDF-TEQ/g) with lower decreases afterwards (to 4.03 pg WHO-PCDD/PCDF-TEQ/g in 2015).

In comparison with the PCDD/PCDF sources relevant in these highly industrialized countries, a different situation was observed in Vietnam. The database for the 1987–1988 period comprises 10 pooled samples from various regions (WHO 1989) showing a wide range of WHO-PCDD/PCDF-TEQ concentrations (median 18.6 pg WHO-PCDD/PCDF-TEQ_{2005}/g, range 6.08 pg/g to 33.9 pg/g). Whereas the lowest equivalent levels outside the European region were reported for the Hanoi area of Vietnam, the highest values were found in certain areas of southern Vietnam. These findings are discussed with regard to the use of the herbicide and defoliant chemical "Agent Orange" during the war in Vietnam in the following subsection "4.3 Asia-Pacific Group". The pooled sample submitted by Vietnam in 2019 and considered to represent the country at that time had 86% lower WHO-PCDD/PCDF-TEQ concentrations than the median of the 1987–1988 concentrations.

4.1 Global Level and Comparison Between UN Regional Groups

For time trend analysis of WHO-PCDD/PCDF-TEQ, results of 274 pooled samples were available. Monitoring of PCDD/PCDF concentrations in two Asian and many European countries PCDD/PCDF covers three decades. The studies in Africa and Latin America and the Caribbean comprised the period 2000–2019 for some countries, whereas most countries participated for the first time in the 2008–2011 period.

In Western European countries with relatively high WHO-PCDD/PCDF-TEQ concentrations in human milk in the first (1987–1988) and second (1992–1993) rounds, a decrease of 51% over 10 years was achieved. Most Eastern European countries participated for the first time in the 1992–1993 period with on average lower WHO-PCDD/PCDF-TEQ concentrations in human milk in this period (median 11.5 pg WHO-PCDD/PCDF-TEQ/g lipid; range 8.3–14.1 pg WHO-PCDD/PCDF-TEQ/g lipid; $N = 7$) than in Western European countries (median 17.1 pg WHO-PCDD/PCDF-TEQ/g lipid; range 10.1–26.0 pg WHO-PCDD/PCDF-TEQ/g lipid; $N = 8$); for the Eastern European group, the decrease within a 10-year-period was 35% (Table 9).

As shown in the following subsections, in comparison with the Western European and Others Group and the Eastern European Group, a higher variation of decrease

Table 9 Overall decrease (%) of WHO-PCDD/PCDF-TEQ concentrations in human milk in the 5 UN Regional Groups and worldwide (computed using all individual samples, see subsection 2.4)

UN Regional Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall[a]
		Theil-Sen method	median method	Theil-Sen method	median method	
Africa	13	10.1	1.1	65.5	10.2	<0.001
Africa without Egypt	12	3.2	0.9	27.4	8.4	<0.001
Asia-Pacific	10	6.0	2.3	45.9	20.5	<0.001
Asia-Pacific without Hong Kong and Vietnam	8	3.2	2.3	27.9	20.5	<0.001
Latin America and Caribbean	9	1.6	4.0	14.7	33.7	<0.001
Latin America and Caribbean without Brazil	8	3.8	4.2	31.9	34.8	<0.001
Eastern Europe	11	4.3	4.6	35.3	37.5	<0.001
Western Europe and Others	14	6.9	6.3	50.9	47.9	<0.001
Global	57	6.3	6.0	47.9	45.9	<0.001
Median of UN Regional Groups	*57*		*4.0*		*33.7*	

[a]For Theil-Sen method

rates between countries was observed in other UN Regional Groups. Lower decrease rates were observed in some countries, but this has to be seen in context with the quite low levels in these countries. Therefore, these regional trends have also to be seen in context with the variation among participating countries. The country-specific aspects are discussed in the following subsections.

Large differences between the decreases calculated by the Theil-Sen method or the median method indicate a considerable variation between countries in a region, e.g. in Africa as a result of the high decrease in Egypt after initially comparably high WHO-PCDD/PCDF-TEQ concentrations in the 2000–2003 period. Furthermore, other African countries participated for the first time in the 2008–2011 period. Therefore, in addition to the overall decrease *(decrease rate constant)* in Africa, also the decrease for African countries without Egypt is given indicating a closer agreement between the Theil-Sen method and the median method. Similarly, for the Asia-Pacific region, the decrease without Hong Kong (excluding a possible influence of the numerous pooled samples sent in two periods) and without Vietnam (excluding a possible influence of the numerous pooled samples in the first period) and in the Group of Latin American and Caribbean Countries the decrease without Brazil (excluding a possible influence of numerous pooled samples sent by Brazil in two sampling periods) were calculated.

The global decrease over 10 years was estimated to be 48% computed by the Theil-Sen method, compared with 46% in the case of the median method. All trends were statistically significant (p-value <0.001).

The exponential trends of WHO-PCDD/PCDF-TEQ concentrations derived by the Theil-Sen method in the five UN Regional Groups and worldwide are illustrated in Fig. 17a–f.

4.2 African Group

Figures 18 (for aggregated data) and 19 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO-PCDD/PCDF-TEQ concentrations in 13 countries from Africa with repeated participation between 2000 and 2019. In Egypt, a reduction of approximately 70% was observed from the median of 9 pooled samples of various regions submitted in the 2000–2003 period to the one pooled sample submitted in the 2016–2019 period, which is considered to represent the country at that time. Most countries participated for the first time in the 2008–2011 period; in these countries, WHO-PCDD/PCDF-TEQ concentrations fell on average by about 10% until the period 2016 to 2019. In seven of these countries with comparably low WHO-PCDD/PCDF-TEQ concentrations (below 3 pg/g lipid), the concentrations remained quite constant over this period. In Ethiopia, the lowest WHO-PCDD/PCDF-TEQ concentrations of all countries in the 2000–2019 studies were found, which remained constant over 7 years (1.01 pg/g in 2012 and 1.02 pg/g in 2019).

The calculated decrease for WHO-PCDD/PCDF-TEQ (Table 10; for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations

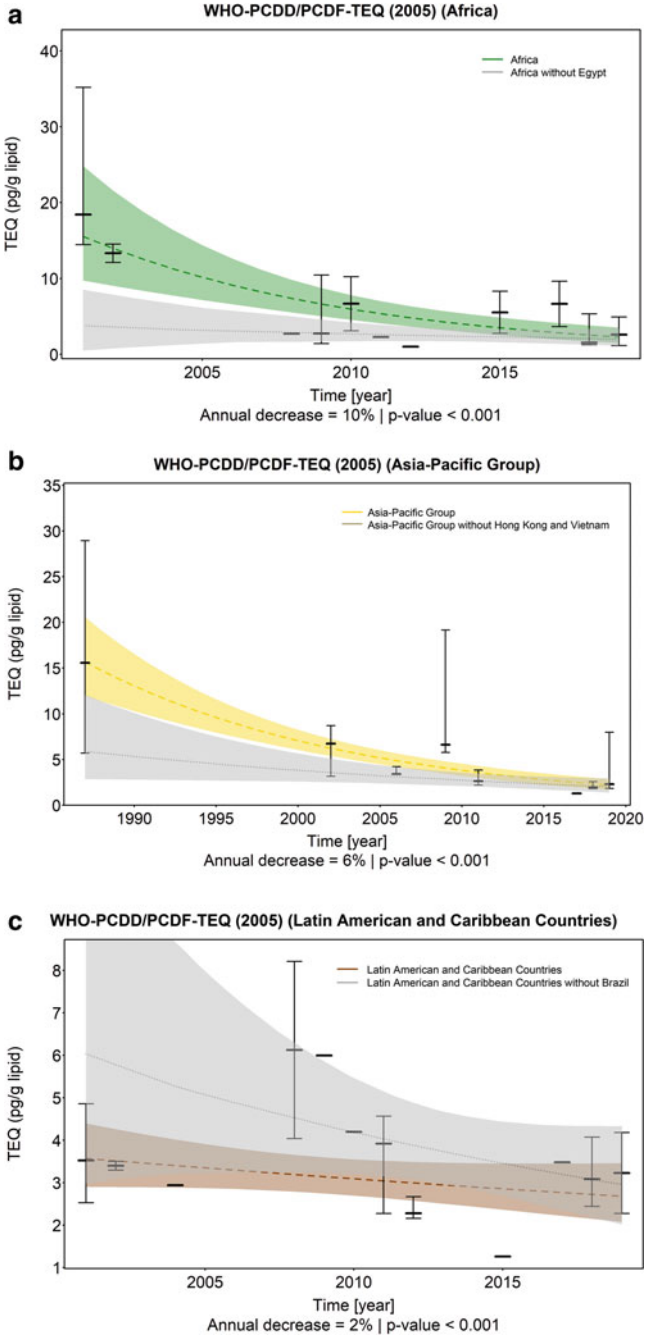

Fig. 17 (**a-f**) Theil-Sen exponential trends of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg WHO-PCDD/PCDF-TEQ_{2005}/g lipid) in the 5 UN regions and worldwide. The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, whiskers show ranges between fifth and 95th percentiles

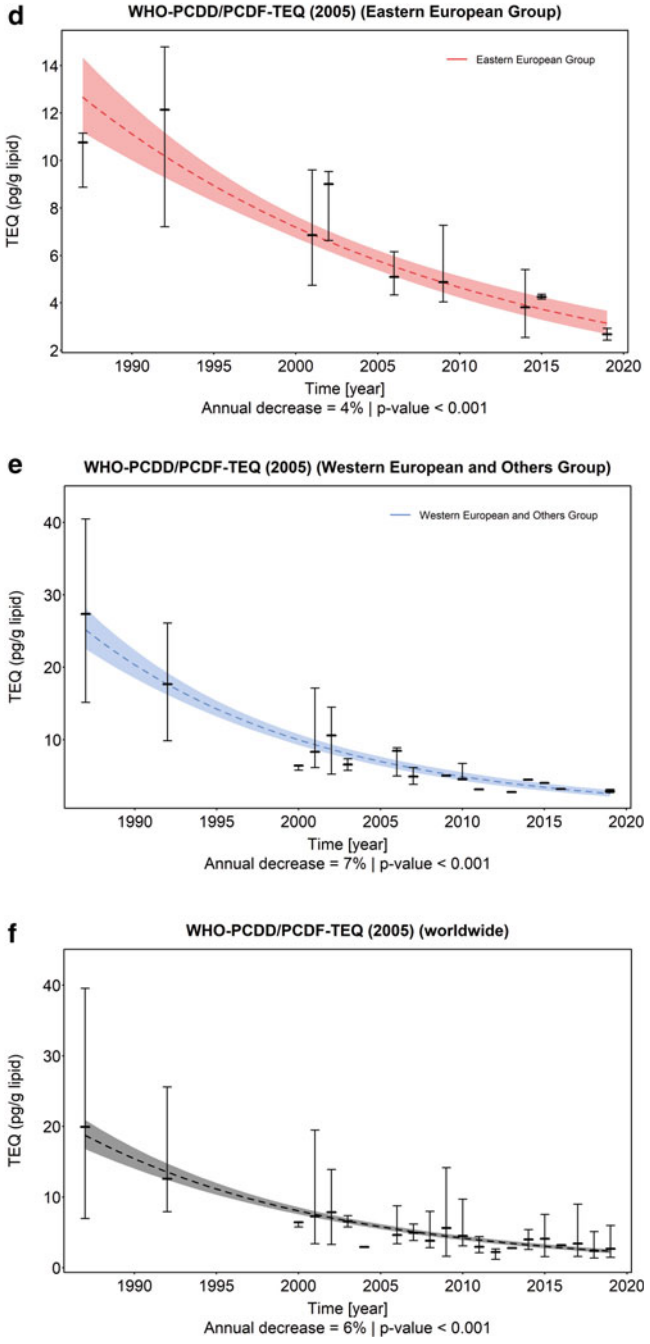

Fig. 17 (continued)

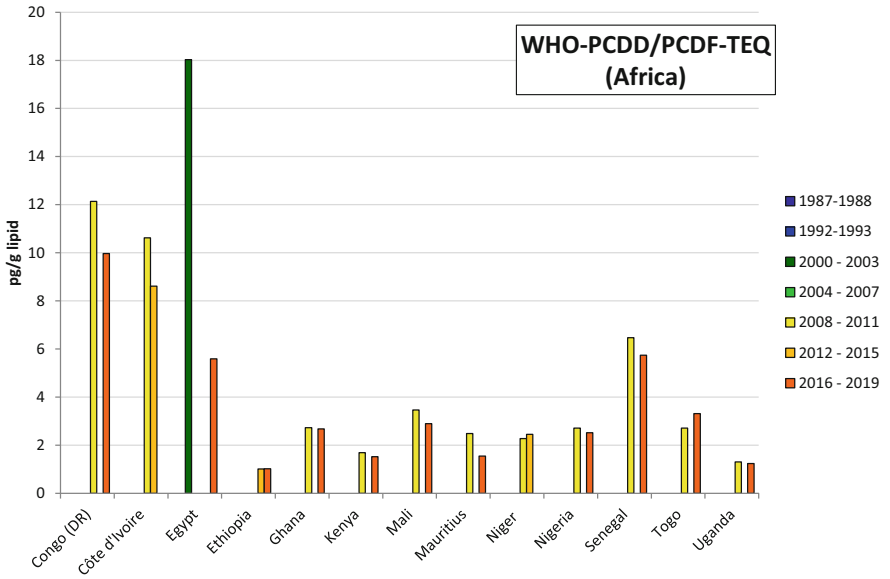

Fig. 18 Overview of the development of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time between 2000 and 2019 for African countries with repeated participation

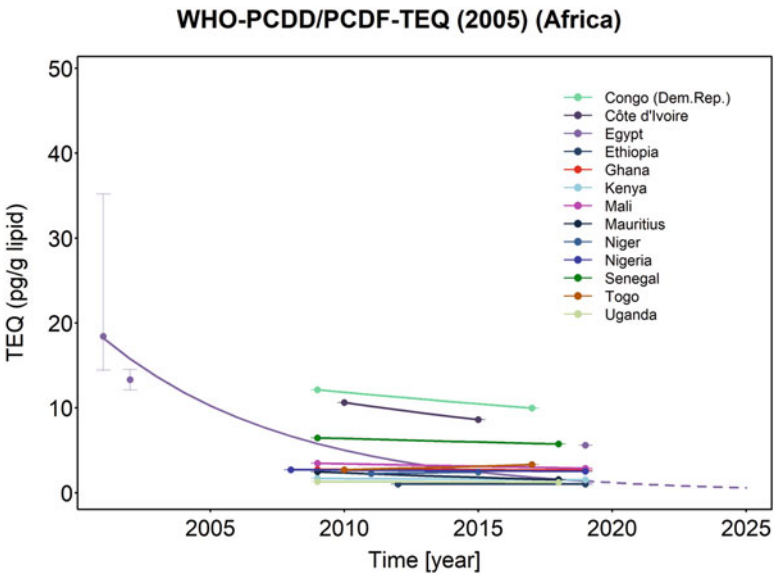

Fig. 19 Temporal tendencies of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid) for African countries with repeated participation between 2000 and 2019 using the Theil-Sen method

Table 10 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk per 1 year, 5 years and 10 years in African countries (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Congo (DR)	2.4	11.6	21.8	1.000
Côte d'Ivoire	4.1	18.9	34.3	1.000
Egypt	13.4	51.2	76.2	<0.001
Ethiopia	−0.1	−0.5	−0.9	1.000
Ghana	0.2	0.9	1.8	1.000
Kenya	1.1	5.2	10.2	1.000
Mali	1.8	8.6	16.5	1.000
Mauritius	5.1	23.2	41.0	1.000
Niger	−1.9	−9.7	−20.3	1.000
Nigeria	0.7	3.3	6.6	1.000
Senegal	1.3	6.4	12.5	1.000
Togo	−2.9	−15.6	−33.5	1.000
Uganda	0.6	3.2	6.3	1.000
Median	1.1	5.2	10.2	

indicating *tendencies*, see subsection 2.4) was higher in case of elevated concentrations as in Egypt, where possible PCDD/PCDF sources for findings in the 2001–2002 samples were discussed (Malisch et al. 2023b). However, downward tendencies seem to level out at ranges below 3 pg WHO-PCDD/PCDF-TEQ/g lipid, which reflects the background contamination.

4.3 Asia-Pacific Group

Figures 20 (for aggregated data) and 21 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO-PCDD/PCDF-TEQ concentrations in 10 countries from the Asia-Pacific Group with repeated participation between 1987 and 2019.

For two Asian countries, data are available already starting in the 1987–1988 round. Since then, the WHO-PCDD/PCDF-TEQ concentration decreased in Thailand by 67% until 2018. As explained above, the database for Vietnam in the 1987–1988 period comprises 10 pooled samples from various provinces (WHO 1989) showing a wide range of WHO-PCDD/PCDF-TEQ concentrations (median 18.6 pg WHO-PCDD/PCDF-TEQ_{2005}/g, range 6.08 pg/g to 33.9 pg/g). Whereas the lowest equivalent levels outside the European region were reported for the Hanoi area of Vietnam, the highest values were found in certain areas of southern Vietnam. The contribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) to the WHO-PCDD/PCDF-TEQ levels in these human milk samples from southern Vietnam was in most cases between about 30% and 66%. This congener was

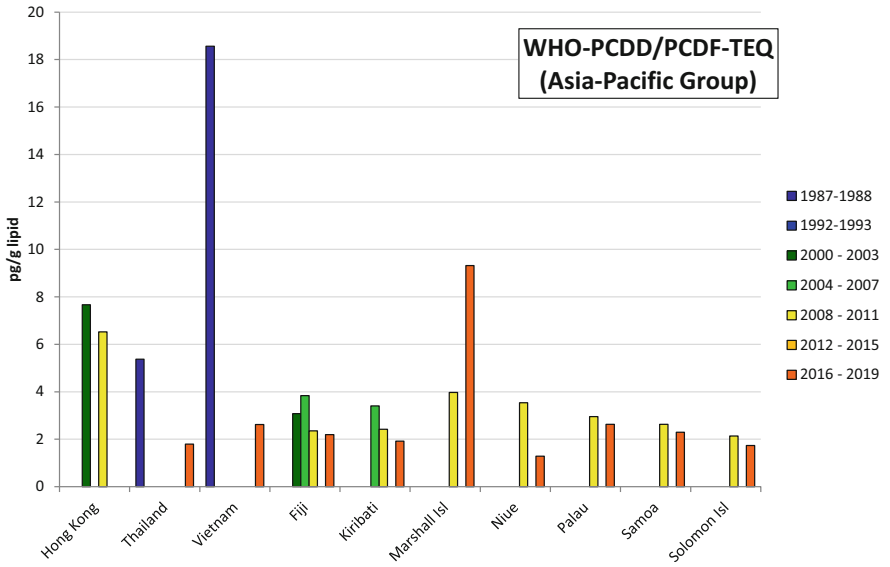

Fig. 20 Overview of the development of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries of the Asia-Pacific Group with repeated participation between 1987 and 2019

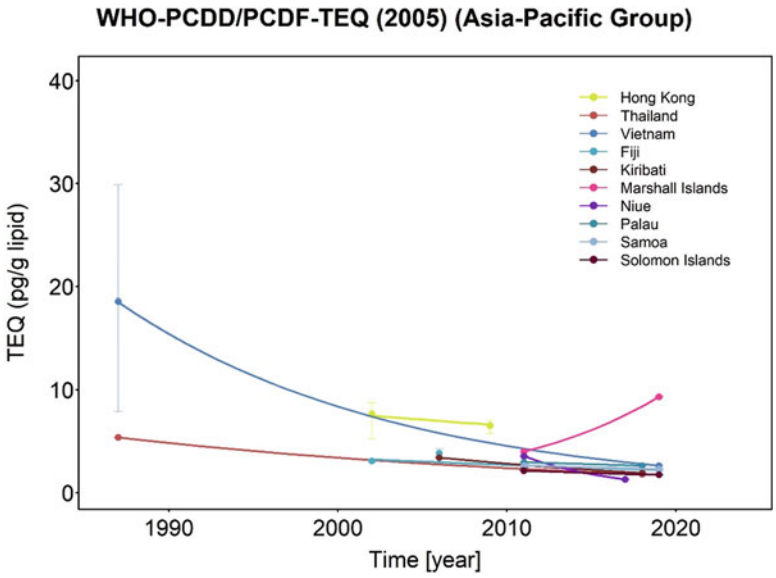

Fig. 21 Temporal tendencies of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid) for countries of the Asia-Pacific Group with repeated participation between 1987 and 2019 using the Theil-Sen method

contained in traces in "Agent Orange", a mixture of two phenoxy herbicides (2,4-dichlorophenoxyacetic acid [2,4-D] and 2,4,5-trichlorophenoxyacetic acid [2,4,5-T]) used by US military as a defoliant chemical during the war in Vietnam (Baughman and Meselson 1973; Schecter et al. 1987; Institute of Medicine (US) 1994; Stellmann et al. 2003). The WHO-PCDD/PCDF-TEQ concentration of pooled samples submitted by Vietnam in 2019, which is considered to represent the country at that time, was 86% lower than the median of the 1987–1988 concentration. The contribution of 2,3,7,8-TCDD to the WHO-PCDD/PCDF-TEQ was 15%—this is within the usual range of all human milk samples of the 2000–2019 periods.

Decreasing temporal trends were observed in nearly all other countries of the Asia-Pacific region. In Hong Kong SAR of China, the median of 13 pooled samples from different subgroups collected in 2002 decreased by 15% to the median of four pooled samples collected in 2009. In this range, the WHO-PCDD/PCDF-TEQ concentrations also decreased in the Pacific countries, which generally had quite low concentrations in the range below 4 pg WHO-PCDD/PCDF-TEQ/g lipid in the period 2008–2011 and below 3 pg WHO-PCDD/PCDF-TEQ/g lipid in the period 2016–2019. Fiji participated in four rounds between 2000 and 2019. Over this period, the WHO-PCDD/PCDF-TEQ concentration decreased by 30% from 3.08 pg/g as an already comparably low level in 2002 to 2.20 pg/g in 2019. Only in the Marshall Islands did the WHO-PCDD/PCDF-TEQ concentration increase from 3.98 pg/g to 9.32 pg/g. For this country, the congener pattern and possible sources for the increased levels were discussed separately (Malisch et al. 2023b).

The overall decreases per 1 year, 5 years and 10 years are given in Table 11 (for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). As concluded above for the decrease in African countries with Egypt as example for elevated concentrations

Table 11 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk per 1 year, 5 years and 10 years in countries of the Asia-Pacific Group (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend p-value overall
Fiji	2.3	10.8	20.5	0.375
Hong Kong	1.8	8.5	16.3	0.002
Kiribati	4.7	21.3	38.0	0.250
Marshall Isl.	−11.2	−70.2	−190	1.000
Niue	15.5	56.9	81.5	1.000
Palau	1.6	7.9	15.2	1.000
Samoa	1.7	8.1	15.5	1.000
Solomon Isl.	2.6	12.2	22.8	1.000
Thailand	3.5	16.2	29.8	1.000
Vietnam	5.9	26.3	45.7	0.002
Median	2.3	10.8	21.7	

related to known sources, a considerable decrease in the levels of POPs within a 10-year period was achieved in Vietnam in comparison with the 1987–1988 samples. Downward tendencies seem to level out at ranges below 3 pg WHO-PCDD/PCDF-TEQ/g lipid, which reflects the background contamination.

4.4 Group of Latin American and Caribbean Countries (GRULAC)

Figures 22 (for aggregated data) and 23 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO-PCDD/PCDF-TEQ concentrations in 9 Latin American and Caribbean countries with repeated participation between 2000 and 2019. In Brazil a reduction of 35% was found from the median of 10 pooled samples of the 2000–2003 period to the median of 3 pooled samples of the 2012–2015 period. Most other countries participated for the first time in the period 2008–2011 with Haiti in 2004–2007. In nearly all countries, a decrease was observed in the following years. In Jamaica, the low levels remained unchanged.

The two samples from Chile apparently showed considerable differences of the WHO-PCDD/PCDF-TEQ concentrations between 2008 (8.44 pg/g) and 2011 (3.92 pg/g). Neither the contribution of PCDD, PCDF and dioxin-like PCB to the TEQ nor the PCDD/PCDF pattern changed during this relatively short time period of 3 years. Thus, sampling differences might explain these findings (Malisch et al. 2023b).

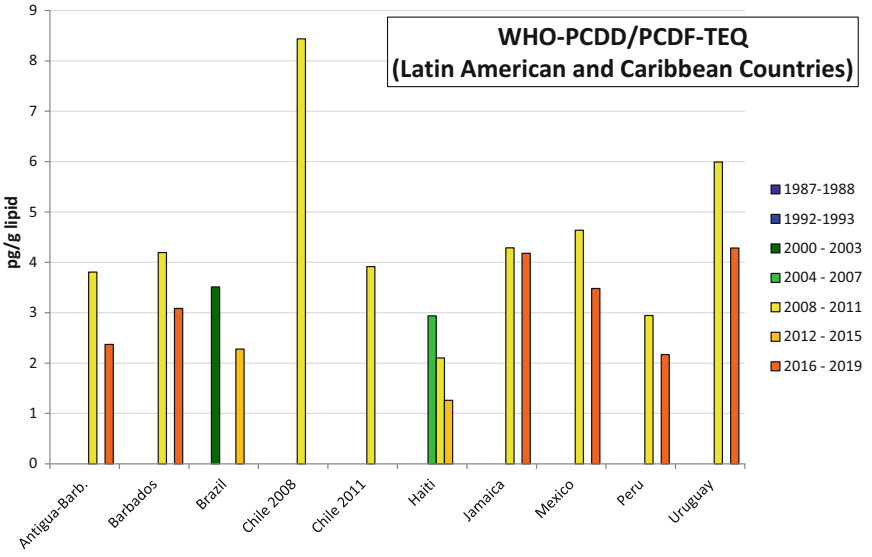

Fig. 22 Overview of the developments of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for Latin American and Caribbean countries with repeated participation between 2000 and 2019

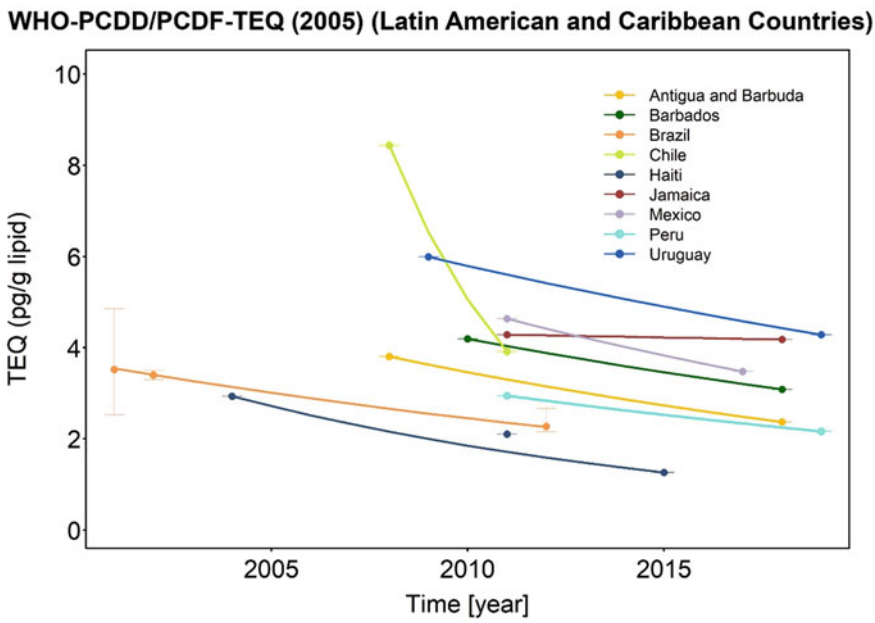

Fig. 23 Temporal tendencies of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid) for Latin American and Caribbean countries with repeated participation between 2000 and 2019 using the Theil-Sen method

Overall decreases per 1 year, 5 years and 10 years are given in Table 12 (for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4).

Table 12 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk per 1 year, 5 years and 10 years in Latin American and Caribbean countries (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Antigua-Barb.	4.6	21.1	37.8	1.000
Barbados	3.8	17.5	31.9	1.000
Brazil	4.0	18.5	33.7	<0.001
Chile	22.6	72.2	92.3	1.000
Haiti	7.4	31.9	53.6	0.250
Jamaica	0.4	1.8	3.5	1.000
Mexico	4.7	21.2	37.9	1.000
Peru	3.8	17.5	31.9	1.000
Uruguay	3.3	15.4	28.5	1.000
Median	4.0	18.5	33.7	

4.5 Eastern European Group

Figures 24 (for aggregated data) and 25 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO-PCDD/PCDF-TEQ concentrations in 11 countries of the Eastern European Group with repeated participation between 1987 and 2019.

One country (Hungary) already participated in the 1987–1988 round. Here, the WHO-PCDD/PCDF-TEQ concentrations decreased by 56% until 2004–2007. In countries which participated for the first time in the 1992–1993 period, the concentrations (as aggregated data, calculated as median if two or more samples were submitted in a certain period) decreased by 77% in Croatia until 2014, by 76% in the Czech Republic until 2019, by 70% in Lithuania until 2015, by 79% in the Slovak Republic until 2019, by 25% in the Ukraine until 2001, and by 22% in Russia until 2001–2002. In two countries participating for the first time in the 2000–2003 period, WHO-PCDD/PCDF-TEQ concentrations decreased by 18% (Bulgaria, 2014) and 22% (Romania, 2014). Finally, two countries (Georgia and Moldova) participated for the first time in the 2008–2011 period. Here, concentrations decreased by 21% (Georgia) and 42% (Moldova) until 2015.

The overall decreases per 1 year, 5 years and 10 years calculated on all individual countries are given in Table 13 (for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). For all Eastern European countries on average the decrease over 10 years of

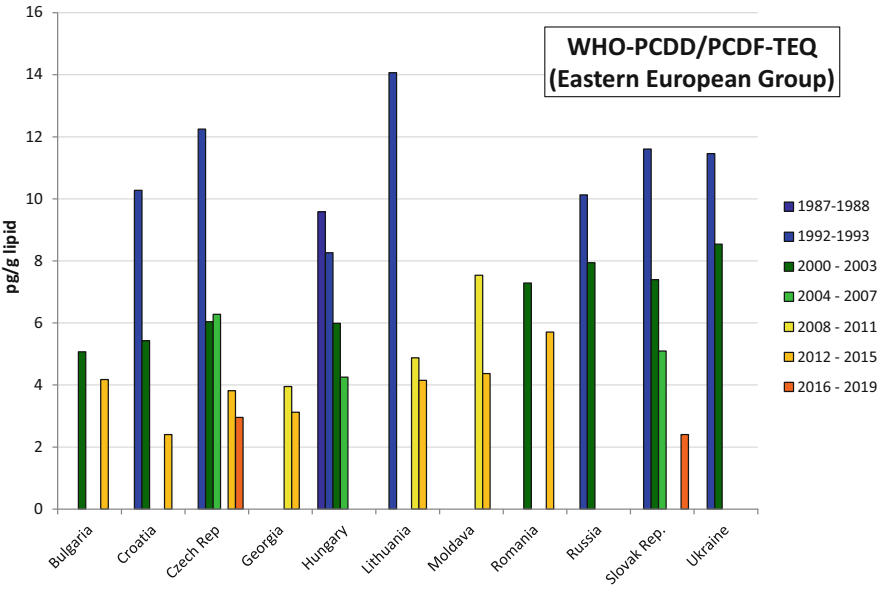

Fig. 24 Overview of the development of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries of the Eastern European Group with repeated participation between 1987 and 2019

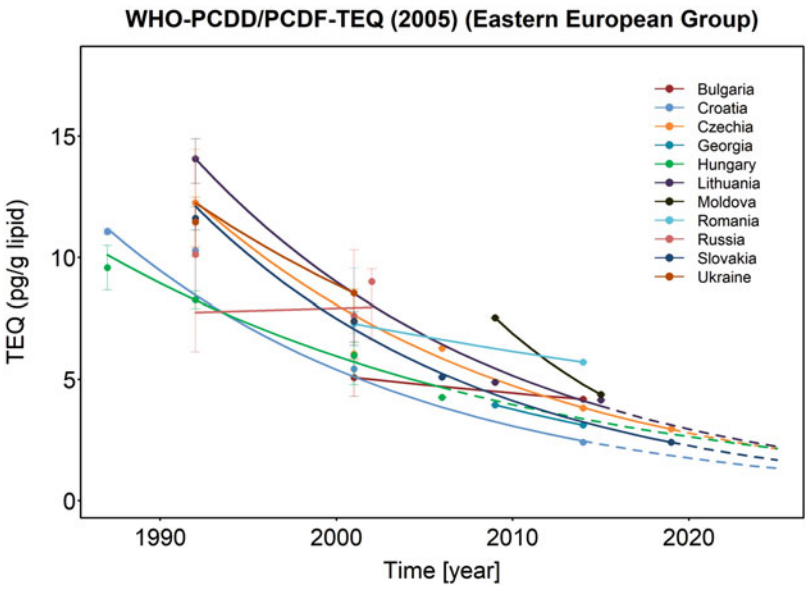

Fig. 25 Temporal tendencies (with some statistically significant time trends) of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid) for countries of the Eastern European Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 13 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk per 1 year, 5 years and 10 years in countries of the Eastern European Group and for four countries, estimated concentrations in 2025 (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Bulgaria	1.5	7.2	13.8		0.250
Croatia	5.5	24.5	43.0	1.2	<0.001
Czechia	5.2	23.3	41.2	2.1	<0.001
Georgia	4.6	21.0	37.5		1.000
Hungary	4.0	18.4	33.5		<0.001
Lithuania	5.4	24.4	42.8	2.2	0.016
Moldova	8.7	36.5	59.7		1.000
Romania	1.9	9.0	17.1		0.250
Russia	−0.3	−1.4	−2.7		1.000
Slovakia	5.8	25.9	45.1	1.7	<0.001
Ukraine	3.9	18.2	33.1		0.031
Median	4.6	21.0	37.5		

35% (calculated by the Theil-Sen method and use of all individual samples) was statistically significant ($p < 0.001$) (see Table 9 in subsection 4.1). Based on a statistically significant decrease and participation also after 2010, a prognosis of the estimated concentrations in 2025 was also derived for 4 countries.

4.6 Western European and Others Group (WEOG)

Figures 26 (for aggregated data) and 27 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO-PCDD/PCDF-TEQ concentrations in 14 countries of the Western European and Others Group with repeated participation between 1987 and 2019. A continuous decrease is observed in all countries over all periods, with high concentrations found in the 1987–1988 period decreasing over the following rounds and later levelling out: Whereas the overall decrease (%) per 10 years before 2000 was 62% and between 2001 and 2010 58%, it was 8% in the decade after 2011 (Table 14).

In Germany, which covers the whole period between 1987 and 2019, based on aggregated data a decrease of WHO-PCDD/PCDF-TEQ concentrations in human milk by 91% was achieved. Similarly successful were Belgium and the Netherlands both with reductions of 89% between the end of the 1980s and the 2012–2015 period.

The median of the decreases for all countries per 10 years was 48% (range 29%–58%). The range between 28% and 54% was statistically significant in

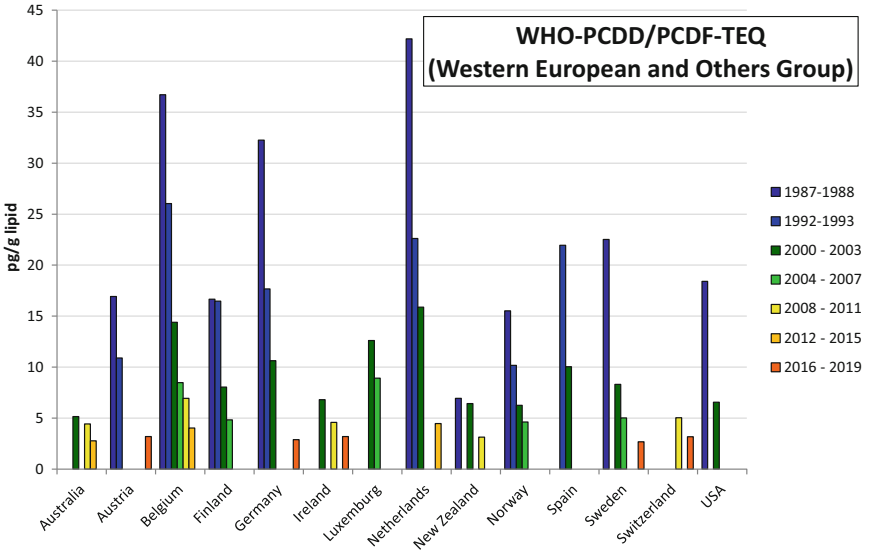

Fig. 26 Overview of the development of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries of the Western European and Others Group with repeated participation between 1987 and 2019

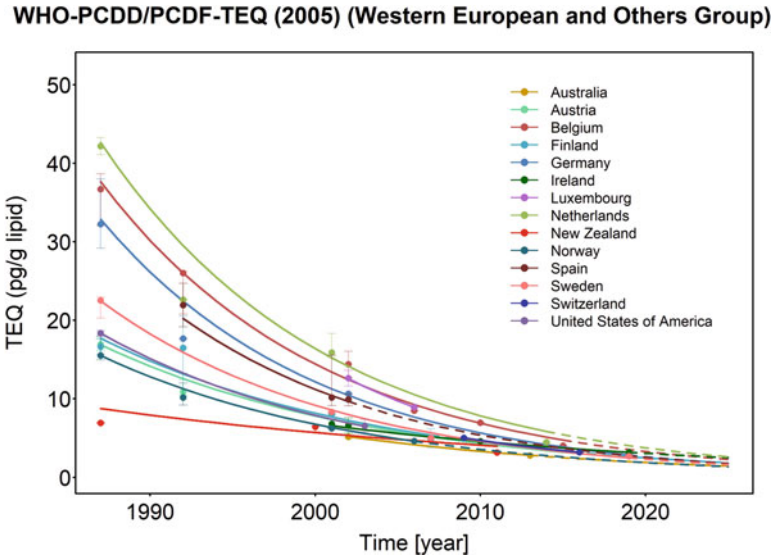

Fig. 27 Temporal tendencies (mostly as statistically significant time trends) of WHO-PCDD/PCDF-TEQ concentrations in human milk (pg/g lipid) for countries of the Western European and Others Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 14 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk in three decades (before 2000; 2001–2010; after 2011) per 1 year, 5 years and 10 years in countries of the Western European and Others Group (calculated by the Theil-Sen method)

Period	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Before 2000	9.2	38.4	62.0	<0.001
2001–2010	8.2	34.9	57.6	<0.001
After 2011	0.8	4.0	7.9	0.121

10 countries (Table 15; for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). For all WEOG countries on average the decrease over 10 years of 51% was statistically significant ($p < 0.001$) (see Table 9 in subsection 4.1). Based on a statistically significant decrease and participation also after 2010, also a prognosis of the estimated concentrations in 2025 was derived for 7 countries.

Table 15 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk per 1 year, 5 years and 10 years and estimated concentrations in 2025 in countries of the Western European and Others Group (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Australia	5.4	24.4	42.8	1.4	0.008
Austria	5.7	25.6	44.7	1.7	0.001
Belgium	7.1	30.9	52.2	1.9	<0.001
Finland	5.8	25.8	44.9		<0.001
Germany	7.4	31.8	53.5	1.6	<0.001
Ireland	3.9	18.2	33.1		0.266
Luxembourg	8.2	34.8	57.5		0.500
Netherlands	7.1	30.8	52.1	2.0	<0.001
New Zealand	3.2	15.2	28.1	2.3	0.016
Norway	6.2	27.4	47.2		<0.001
Spain	7.1	30.8	52.2		0.003
Sweden	6.6	29.1	49.7	1.4	<0.001
Switzerland	6.4	28.0	48.1		1.000
USA	6.3	27.7	47.7		0.125
Median	6.3	27.8	47.9		

5 Total Toxic Equivalents of PCDD, PCDF and Dioxin-Like PCB (WHO_{2005}-TEQ)

All TEQ-related evaluations are based on the WHO-TEFs as proposed in 2005 (Van den Berg et al. 2006). The term "WHO-PCDD/PCDF-TEQ" is used for TEQ of PCDD/PCDF, "WHO-PCB-TEQ" for TEQ of dioxin-like PCB and "WHO_{2005}-TEQ" for the total TEQ as sum of the contribution of PCDD/PCDF and dioxin-like PCB. Results are given as upper bound concentrations and expressed on a lipid basis (Malisch et al. 2023b).

As dioxin-like PCB were of interest starting from the second WHO round (1992–1993), time trends for total TEQ (WHO_{2005}-TEQ) can be derived only beginning with this period. For a first general estimation of time trends, 130 aggregated data for 54 countries with repeated participation in the period 1992–2019 and 116 aggregated data for 50 countries for the period 2000–2019 were used as the basis. In 6 periods between 1992 and 2019, the median of the concentrations decreased considerably from 21.7 pg WHO_{2005}-TEQ/g to 3.91 pg/g (= 82% decrease). The Stockholm Convention was adopted in 2001 and entered into force in 2004. Therefore, if only the period 2000–2019 is taken into consideration, the median concentrations went down by 70% from initially 13.1 pg WHO_{2005}-TEQ/g in the period 2000–2003. Other than observed for the continuous decrease of WHO-PCDD/PCDF-TEQ levels, the WHO_{2005}-TEQ concentrations seemed to decrease with a fluctuation by an increase from the fifth to the sixth round

Table 16 Ranges of total TEQ concentrations in human milk (pg WHO_{2005}-TEQ/g lipid) in 6 periods between 1992 and 2019 for countries with two or more participations and decrease of the median concentrations calculated as % of round 2 (1992–1993) and round 3 (2000–2003), respectively, as point of reference (aggregated data using the median if two or more samples were submitted by a country in a certain period; ND = not determined)

			WHO_{2005}-TEQ			Decrease of median over time	
Round	Period	No of countries/ participations	Min	Median	Max	% relative to round 2	% relative to round 3
1	1987–1988	–	ND	ND	ND		–
2	1992–1993	14	9.57	21.7	33.9	100%	–
3	2000–2003	23	4.42	13.1	23.0	60%	100%
4	2004–2007	11	5.06	7.80	15.7	36%	59%
5	2008–2011	36	2.01	5.62	15.0	26%	43%
6	2012–2015	15	1.54	6.50	11.1	30%	50%
7	2016–2019	31	1.29	3.91	11.6	18%	30%
	1992–2019	130	1.29	6.53	33.9		
	2000–2019	116	1.29	6.04	23.0		

(Table 16). As explained above for non-dioxin-like-PCB, also these changes are the result of participation of different countries in different rounds. Therefore, for more precise time trends, a country-specific evaluation is necessary.

5.1 Global Level and Comparison Between UN Regional Groups

Figure 28 illustrates the developments in 54 countries from all UN regions with repeated participation between 1992 and 2019 for 130 aggregated data. Similar temporal trends as described for WHO-PCDD/PCDF-TEQ were observed. Discrepancies depend on dioxin-like PCB and their contribution to total TEQ in comparison with the PCDD/PCDF contribution (Malisch et al. 2023b).

For time trend analysis of total TEQ concentrations, results of 226 individual pooled samples were available. Monitoring of WHO_{2005}-TEQ concentrations in many European countries PCDD/PCDF covers three decades, whereas the studies in Africa, the Asia-Pacific Region, Latin America and the Caribbean comprised the period 2000–2019, with most countries participating for the first time in the 2008–2011 period.

In nearly all countries, WHO_{2005}-TEQ concentrations were consistently decreasing over the whole monitored period. A 50% decrease in the levels of total TEQ within a 10-year period was achieved in regions with countries with initially relatively high WHO_{2005}-TEQ concentrations in human milk, as found, e.g., in Western European in the second (1992–1993) or third (2000–2003) round or in an African country in the 2000–2003 period. In comparison with the initial levels found in Western European countries, most Eastern European countries had on average lower WHO_{2005}-TEQ concentrations in human milk; for this group, the decrease within a 10-year-period was nearly 40%. The lower decrease rates observed in some

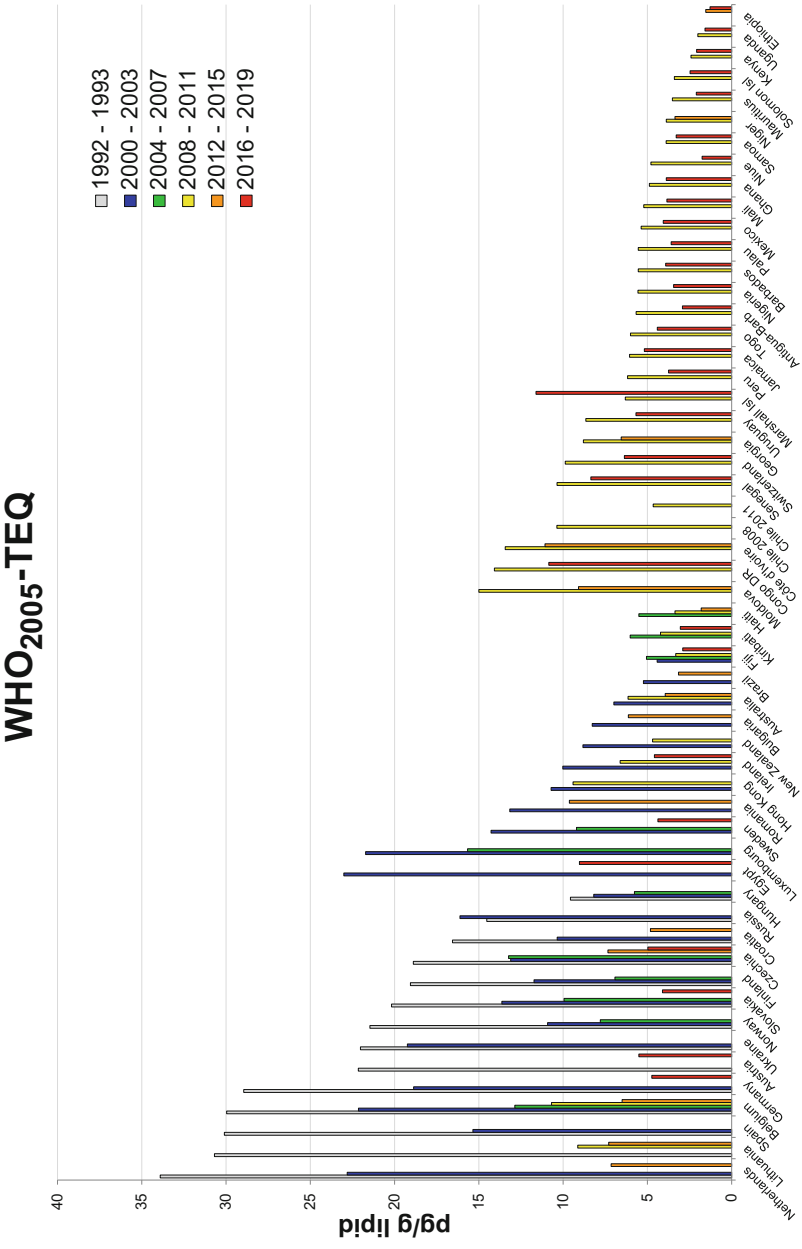

Fig. 28 Overview of the development of total TEQ (WHO_{2005}-TEQ) concentrations in human milk (pg/g lipid) over time between 1992 and 2019 for 54 countries from all UN regions with two or more participations

Table 17 Overall decrease (%) of WHO_{2005}-TEQ concentrations in human milk in the 5 UN Regional Groups and worldwide (computed using all individual samples, see subsection 2.4)

UN Regional Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years]		Trend *p*-value overall [a]
		Theil-Sen method	median method	Theil-Sen method	median method	
Africa	13	10.1	3.2	65.4	27.7	<0.001
Africa without Egypt	12	4.8	3.1	39.0	27.0	<0.001
Asia-Pacific	8	6.6	3.3	49.3	28.3	<0.001
Asia-Pacific without Hong Kong	7	3.3	3.9	28.6	32.8	<0.001
Latin America and Caribbean	9	2.2	4.6	19.6	37.3	<0.001
Latin America and Caribbean without Brazil	8	4.3	5.3	35.3	41.9	<0.001
Eastern Europe	11	4.3	4.7	35.6	38.3	<0.001
Western Europe and Others	13	6.4	6.4	48.5	48.1	<0.001
Global	54	6.6	6.4	49.5	48.5	<0.001
Median of UN Regional Groups			*4.6*		*37.3*	

[a] for Theil-Sen method

African, Pacific, Latin American and Caribbean and Eastern European countries have to be seen in context with the quite low initial levels in these countries in comparison with some countries of other regions and their periods of participation.

The regional trends (Table 17) have also to be seen in context with the variation among participating countries. Large differences between the decreases calculated by the Theil-Sen method or the median method indicate a considerable variation between countries in a region, e.g., in Africa as result of the high decrease in Egypt after initially comparatively high WHO-PCDD/PCDF-TEQ concentrations in the 2000–2003 period. The decreases calculated by the Theil-Sen method for the African Group without Egypt are in better agreement with the median method. Similarly, in Latin America and the Caribbean, a differentiation between Brazil (with participation already in the 2000–2003 period and submission of 10 pooled samples at that time) and other countries (mostly participating for the first time in the period 2008–2011 with submission of one pooled sample per period) was introduced. In the Asia-Pacific Group, the decrease (*decrease rate constant*) without Hong Kong was also calculated to exclude a possible influence of the comparably high number of pooled samples from different subgroups collected in 2002 and 2009. The country-specific aspects are discussed in the following sections.

The annual global decrease was estimated at 6.6% (i.e. half-life of 10.1 years) computed by the Theil-Sen method, which is in agreement with an annual decrease of 6.4% in the case of the median method. All trends were statistically significant (p-value <0.001).

The exponential trends of WHO_{2005}-TEQ concentrations derived by the Theil-Sen method in the five UN Regional Groups and worldwide are illustrated in Fig. 29a–f.

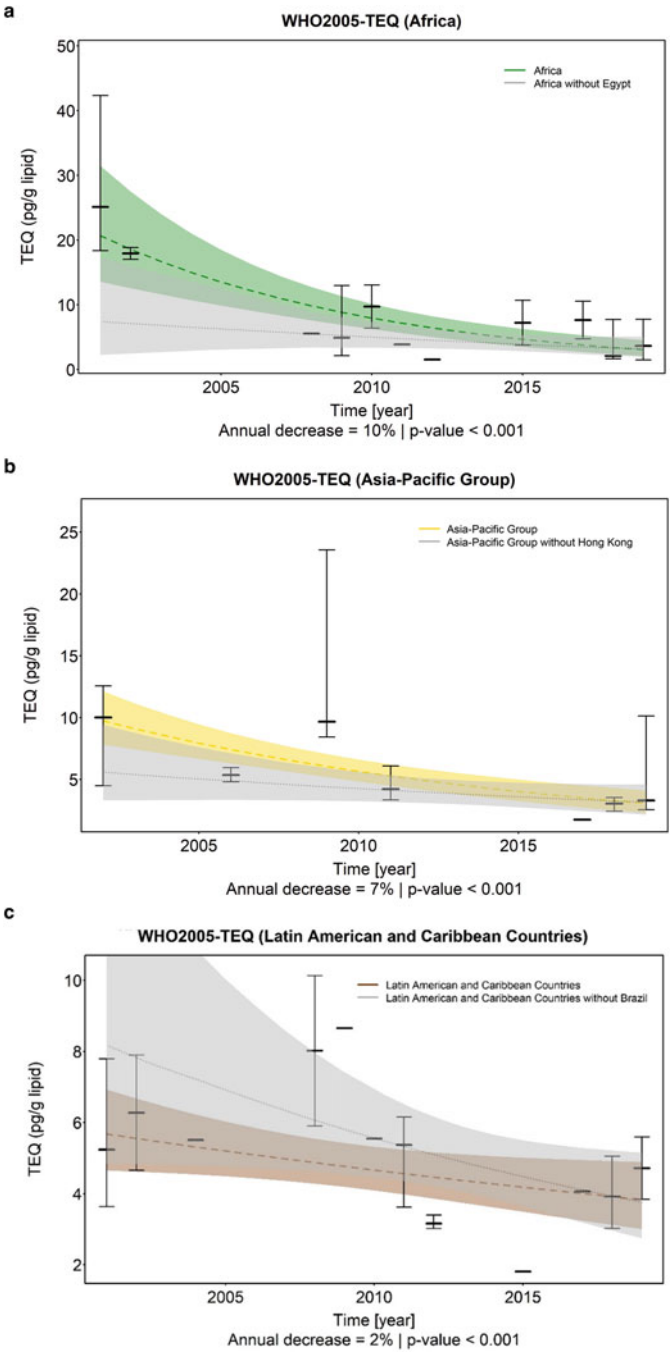

Fig. 29 (**a-f**) Theil-Sen exponential trends of total TEQ concentrations in human milk (pg WHO_{2005}-TEQ/g lipid) in the 5 UN regions and worldwide. The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, whiskers show ranges between fifth and 95th percentiles

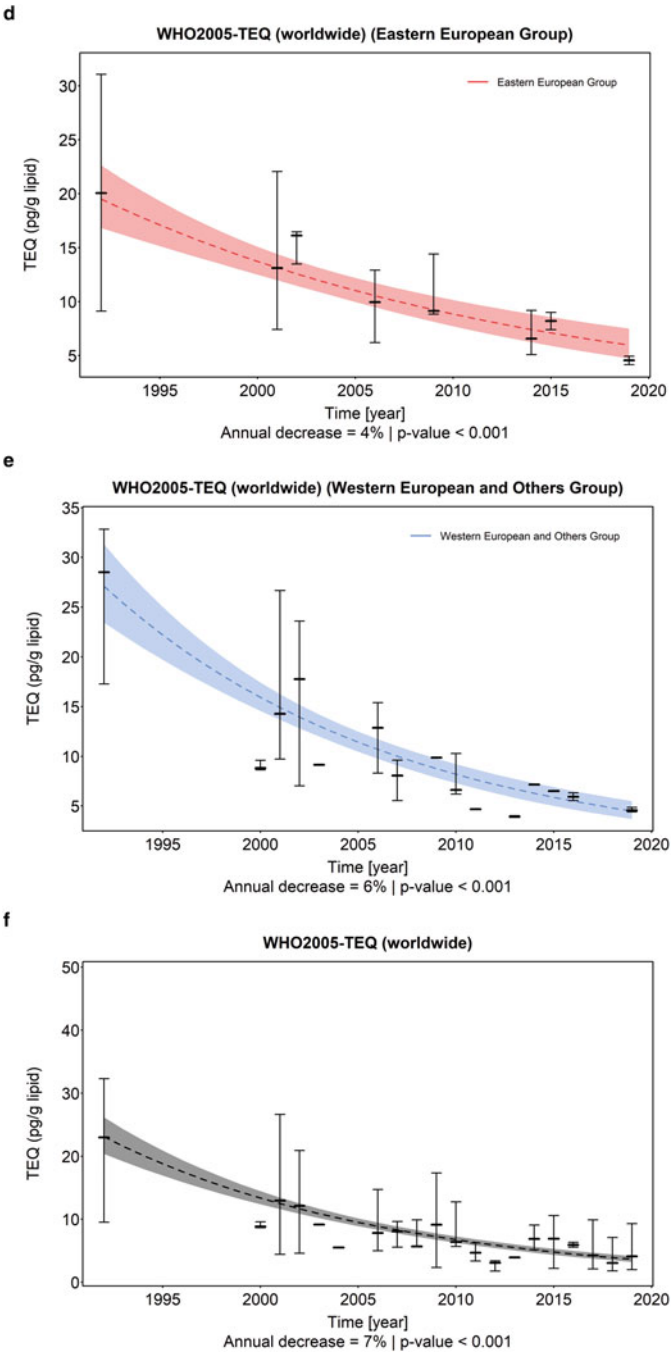

Fig. 29 (continued)

5.2 African Group

Figures 30 (for aggregated data) and 31 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO_{2005}-TEQ concentrations in 13 countries from the Africa Group with repeated participation between 2000 and 2019.

Most countries participated for the first time in the 2008–2011 period; in these countries, WHO_{2005}-TEQ concentrations fell on average by about 23% (range 14%–40%) until the period 2016 to 2019. The calculated decrease for WHO_{2005}-TEQ within a 10-year period is in a range between 14% and 61% (median 28%) (Table 18; for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). The highest rate was observed in Egypt, where PCDD/PCDF sources were discussed for elevated findings in the 2001–2002 samples (Malisch et al. 2023b).

For the decrease of total TEQ concentrations, the relative contribution of dioxin-like PCB and PCDD/PCDF has an influence. As an example for changes of the contribution of dioxin-like PCB to the total TEQ over time, in Togo the relatively low background concentrations for WHO-PCDD/PCDF-TEQ increased from 2008–2011 to 2016–2019 by 22%, whereas the WHO_{2005}-TEQ concentration decreased by 26%. In this period, the contribution of dioxin-like PCB to the total TEQ decreased in Togo from 55% in 2010 to 25% in 2017 (Malisch et al. 2023b).

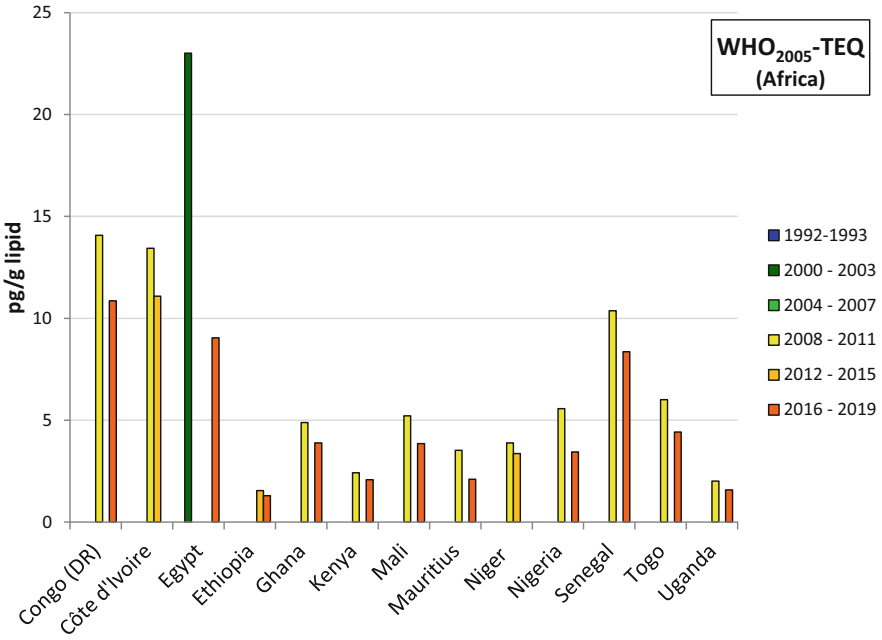

Fig. 30 Overview of the development of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for African countries with repeated participation between 2000 and 2019

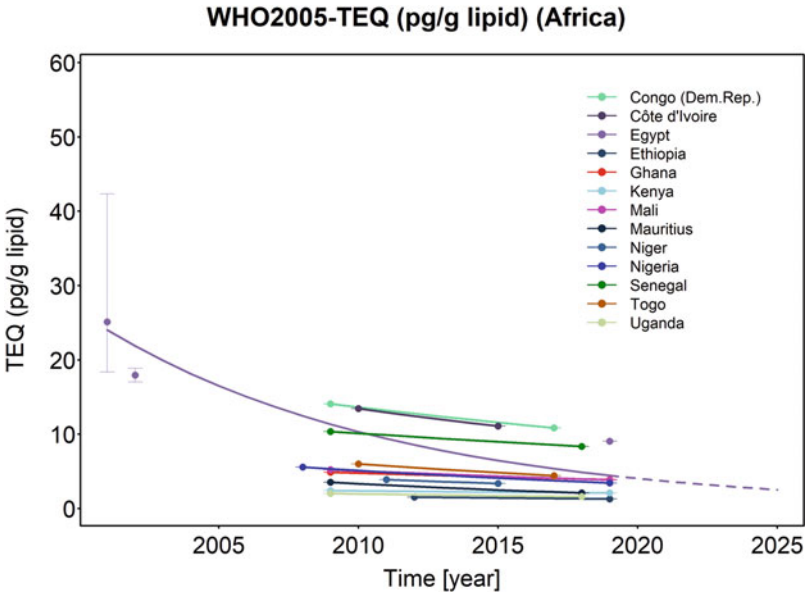

Fig. 31 Temporal tendencies of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid) for African countries with repeated participation between 2000 and 2019 using the Theil-Sen method

Table 18 Overall decrease (%) of WHO_{2005}-TEQ in human milk per 1 year, 5 years and 10 years in African countries and for one country, estimated concentrations (pg WHO_{2005}-TEQ/g lipid) in 2025 (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Congo (DR)	3.2	15.0	27.7	1.000
Côte d'Ivoire	3.8	17.5	32.0	1.000
Egypt	9.0	37.5	60.9	<0.001
Ethiopia	2.5	12.0	22.5	1.000
Ghana	2.3	10.9	20.6	1.000
Kenya	1.5	7.2	13.9	1.000
Mali	3.0	14.1	26.3	1.000
Mauritius	5.6	25.0	43.8	1.000
Niger	3.5	16.4	30.2	1.000
Nigeria	4.3	19.6	35.3	1.000
Senegal	2.4	11.3	21.2	1.000
Togo	4.3	19.7	35.5	1.000
Uganda	2.6	12.4	23.2	1.000
Median	3.2	15.0	27.7	

Whereas the WHO-PCDD/PCDF-TEQ concentrations decreased in African countries from 2008–2011 to 2016–2019 on average by 10% (range 38% decrease to 22% increase), the WHO-PCB-TEQ concentrations decreased on average by 47% (range 23% to 67%).

5.3 Asia-Pacific Group

Figures 32 (for aggregated data) and 33 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO_{2005}-TEQ concentrations in 8 countries from the Asia-Pacific Group with repeated participation between 1992 and 2019.

Decreasing temporal trends were observed in nearly all other countries of the Asia-Pacific region. In Hong Kong SAR of China, the median of 13 pooled samples from different subgroups collected in 2002 decreased by 12% to the median of four pooled samples collected in 2009. Fiji participated in four rounds between 2000 and 2019. Over this period, the WHO_{2005}-TEQ concentration decreased by 34% from 4.42 pg/g (as the median of two pooled samples) as an already comparably low level in 2002 to 2.90 pg/g in 2019. Only in the Marshall Islands the WHO_{2005}-TEQ concentration increased from 6.32 pg/g in 2011 to 11.6 pg/g in 2019. For this

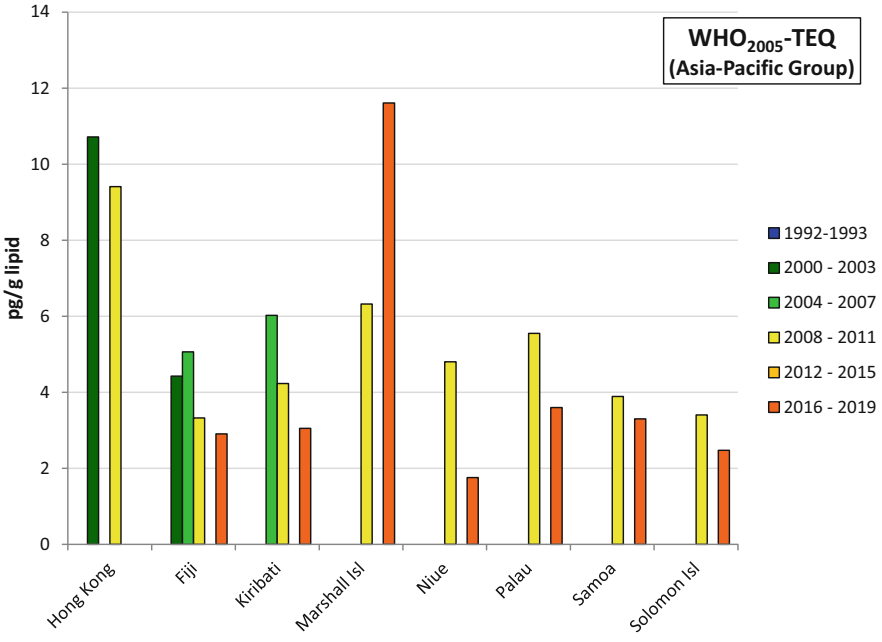

Fig. 32 Overview of the development of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries of the Asia-Pacific Group with repeated participation between 1987 and 2019

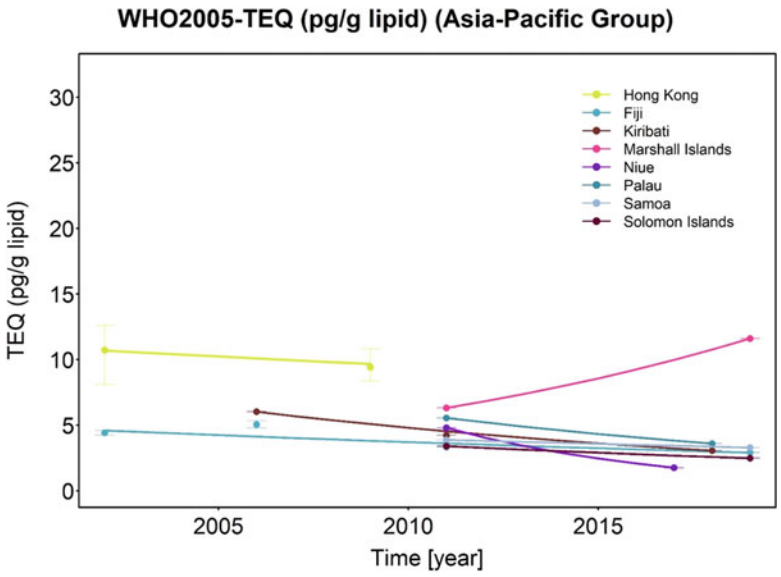

Fig. 33 Temporal tendencies of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid) for countries of the Asia-Pacific Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 19 Overall decrease (%) of WHO_{2005}-TEQ in human milk per 1 year, 4 years, 5 years and 10 years in countries of the Asia-Pacific Group (calculated by the Theil-Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 4 years	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Hong Kong	1.4	5.6	6.9	13.3	0.003
Fiji	2.7	10.2	12.6	23.6	0.191
Kiribati	5.5	20.3	24.7	43.3	0.250
Marshall Islands	−7.9	−35.5	−46.3	−113.9	1.000
Niue	15.4	48.8	56.7	81.3	1.000
Palau	6.0	21.9	26.6	46.2	1.000
Samoa	2.0	7.9	9.8	18.6	1.000
Solomon Islands	3.9	14.7	18.0	32.8	1.000
Median	3.3	12.5	15.3	28.3	

country, the congener pattern and possible sources for the increased levels were discussed separately (Malisch et al. 2023b).

The overall decreases per 1 year, 5 years and 10 years are given in Table 19 (for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). The decrease in the levels of WHO_{2005}-TEQ within a 10-year period was in nearly all countries between 13%

and 81% showing a continuous decrease also in the range of low background concentrations.

5.4 Group of Latin American and Caribbean Countries (GRULAC)

Figures 34 (for aggregated data) and 35 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO_{2005}-TEQ concentrations in 9 Latin American and Caribbean countries with repeated participation between 2000 and 2019. In Brazil a reduction of 40% was found from the median of 10 pooled samples of the 2000–2003 period to the median of 3 pooled samples of the 2012–2015 period. Most other countries participated for the first time in the period 2008–2011, Haiti in 2004–2007. In all countries, a decrease was observed in the following years.

The overall decreases per 1 year, 5 years and 10 years are given in Table 20 (for statistical significance of *trends* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). The decrease in the levels of WHO_{2005}-TEQ within a 10-year period was in all countries between 20% and 93% showing a continuous decrease also in the range of low background concentrations. The high decrease found in Chile for samples collected over a

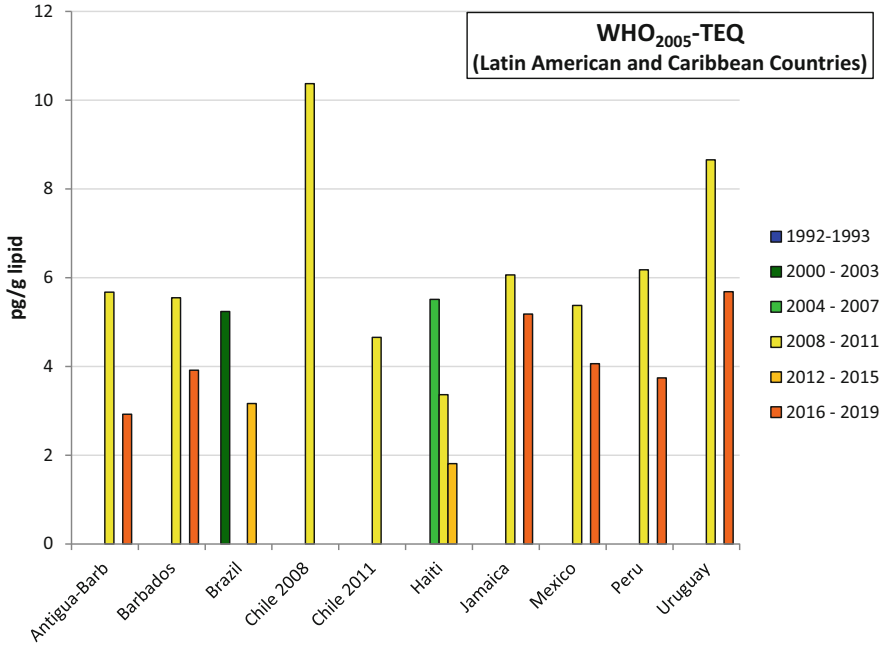

Fig. 34 Overview of the development of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for Latin American and Caribbean countries with repeated participation between 2000 and 2019

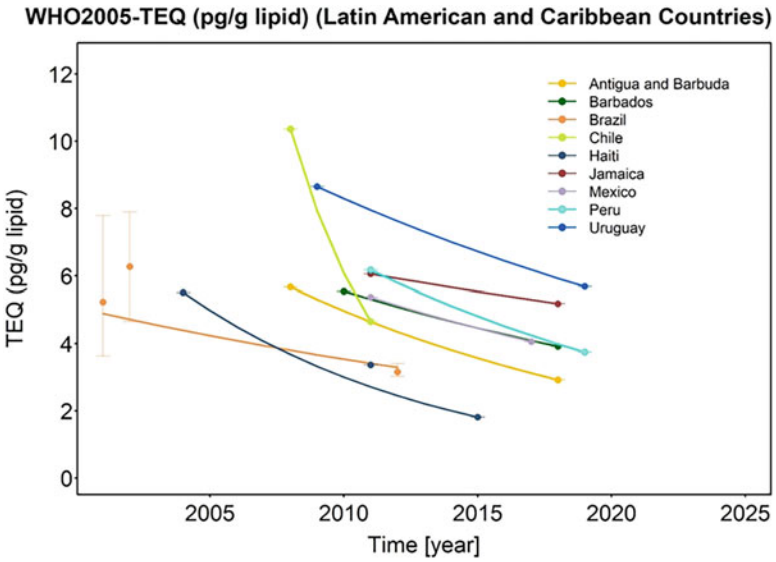

Fig. 35 Temporal tendencies of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid) for Latin American and Caribbean countries with repeated participation between 2000 and 2019 using the Theil-Sen method

Table 20 Overall decrease (%) of WHO_{2005}-TEQ in human milk per 1 year, 5 years and 10 years in Latin American and Caribbean countries (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Antigua-Barb.	6.4	28.2	48.5	1.000
Barbados	4.3	19.6	35.3	1.000
Brazil	3.5	16.5	30.2	0.056
Chile	23.4	73.6	93.1	1.000
Haiti	9.6	39.7	63.6	0.250
Jamaica	2.2	10.6	20.1	1.000
Mexico	4.6	20.8	37.3	1.000
Peru	6.1	26.9	46.5	1.000
Uruguay	4.1	18.9	34.3	1.000
Median	4.6	20.8	37.3	

relatively short period (2008 and 2011) has to be seen in context with discussion of PCDD/PCDF patterns and the assumption that differences in the regional origin might explain these findings (Malisch et al. 2023b).

5.5 Eastern European Group

Figures 36 (for aggregated data) and 37 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO_{2005}-TEQ concentrations in 11 countries of the Eastern European Group with repeated participation between 1987 and 2019.

In countries which participated for the first time in the 1992–1993 period, the concentrations (as aggregated data, calculated as median if two or more samples were submitted in a certain period) decreased by 71% in Croatia until 2014, by 74% in the Czech Republic until 2019, by 40% in Hungary until 2006, by 76% in Lithuania until 2015, by 80% in the Slovak Republic until 2019, by 13% in the Ukraine until 2001, and increased by 11% in Russia until 2001–2002. In two countries participating for the first time in the 2000–2003 period, WHO_{2005}-TEQ concentrations decreased by 26% (Bulgaria, 2014) and 27% (Romania, 2014). Finally, two countries (Georgia and Moldova) participated for the first time in the 2008–2011 period, where concentrations decreased in both by 39% until 2015.

The overall decreases per 1 year, 4 years, 5 years and 10 years calculated for all individual countries are given in Table 21 (for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). Statistically significant overall decreases per 10 years for countries between 14% and 47% were found in 6 countries. For four of these with participation also after 2010, also a prognosis of the estimated concentrations in 2025 was derived.

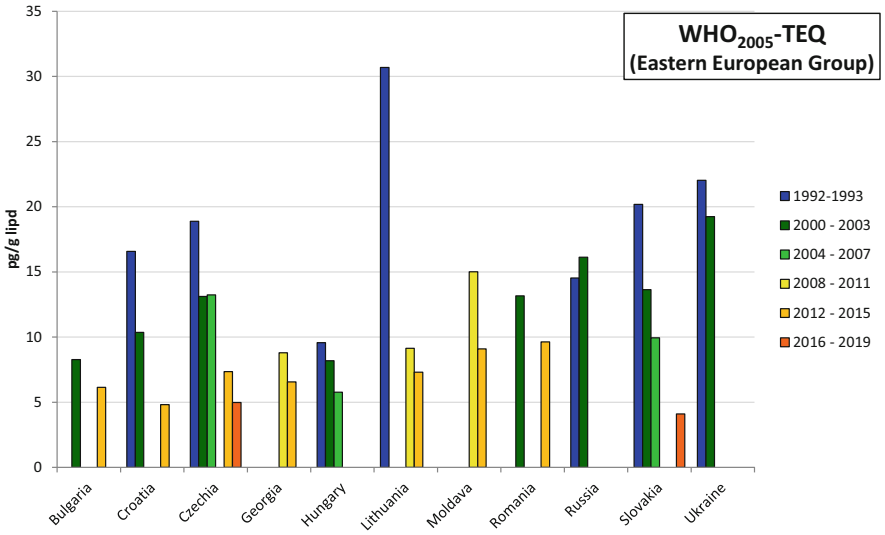

Fig. 36 Overview of the development of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries of the Eastern European Group with repeated participation between 1987 and 2019

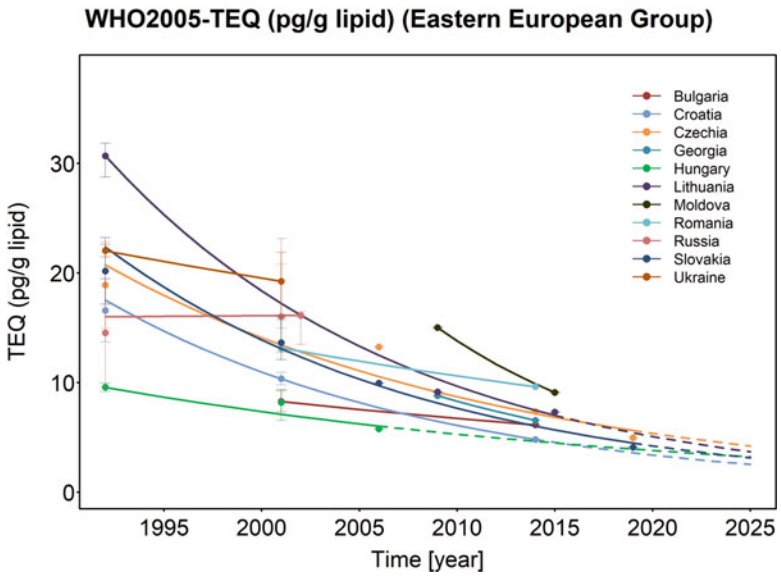

Fig. 37 Temporal tendencies (with statistically significant time trends for 6 countries) of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid) for countries of the Eastern European Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 21 Overall decrease (%) of WHO_{2005}-TEQ in human milk per 1 year, 5 years and 10 years in countries of the Eastern European Group and for four countries, estimated concentrations (pg WHO_{2005}-TEQ/g lipid) in 2025 (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Bulgaria	2.3	10.9	20.6		0.250
Croatia	5.7	25.4	44.4	2.5	0.008
Czechia	4.7	21.4	38.3	4.1	<0.001
Georgia	5.7	25.5	44.5		1.000
Hungary	3.2	15.2	28.0		0.002
Lithuania	6.2	27.5	47.4	3.5	0.016
Moldova	8.0	34.2	56.7		1.000
Romania	2.4	11.3	21.4		0.250
Russia	−0.1	−0.4	−0.8		0.394
Slovakia	5.8	25.7	44.9	2.9	<0.001
Ukraine	1.5	7.2	13.9		0.002
Median	4.7	21.4	38.3		

5.6 Western European and Others Group (WEOG)

Figures 38 (for aggregated data) and 39 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.4) illustrate the time trends of WHO_{2005}-TEQ concentrations in 13 countries of the Western European and Others Group with repeated participation between 1992 and 2019. A continuous decrease is observed in all countries over all periods, with high concentrations found in the 1987–1988 period decreasing faster over the following rounds and later at lower concentration ranges slower: Whereas the overall decrease (%) per 10 years before 2000 was 75% and 58% between 2001 and 2010, it was 8% in the decade after 2011 (Table 22).

Based on aggregated data, in Germany, which covers the whole period between 1992 and 2019, a decrease of WHO_{2005}-TEQ concentrations in human milk by 84% was achieved. Similarly successful were Belgium and the Netherlands with reductions of nearly 80% between 1992 and the 2012–2015 period.

The median of the decreases *(decrease rate constants)* for all countries per 10 years was 48% (range 34–56%) (Table 23; for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.4). Based on statistically significant decrease rates, estimated concentrations in 2025 were derived for 4 countries with participation also after 2010.

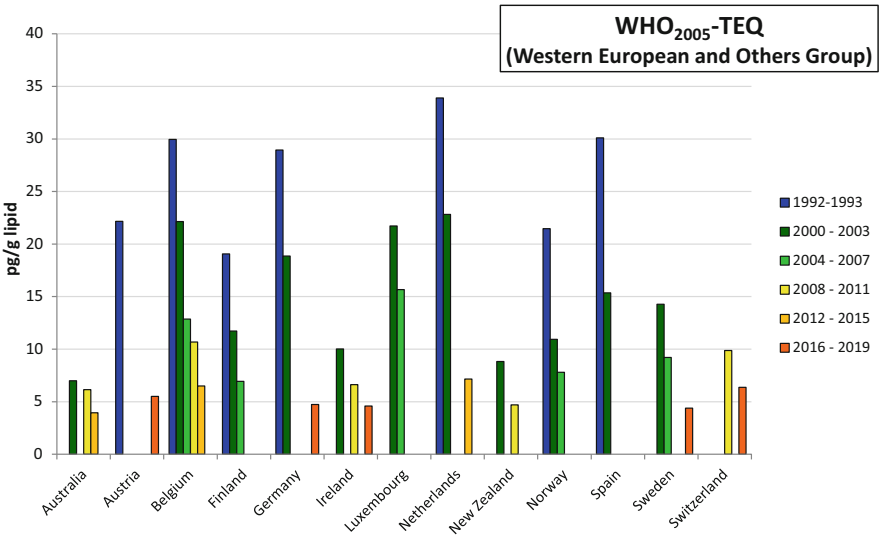

Fig. 38 Overview of the development of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid; aggregated data) over time for countries of the Western European and Others Group with repeated participation between 1987 and 2019

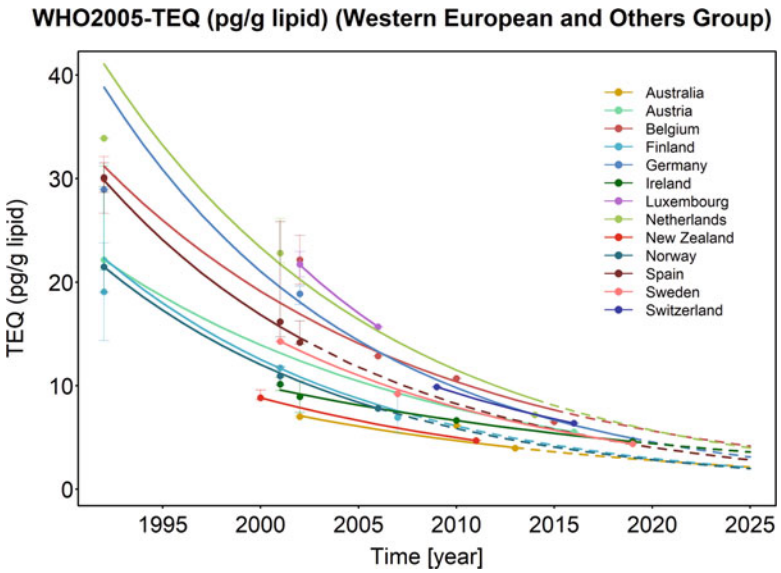

Fig. 39 Temporal tendencies (with many statistically significant time trends) of WHO_{2005}-TEQ concentrations in human milk (pg/g lipid) for countries of the Western European and Others Group with repeated participation between 1987 and 2019 using the Theil-Sen method

Table 22 Overall decrease (%) of WHO-PCDD/PCDF-TEQ in human milk in three decades (before 2000; 2001–2010; after 2011) per 1 year, 5 years and 10 years in countries of the Western European and Others Group (calculated by the Theil-Sen method)

Period	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Trend *p*-value overall
Before 2000	13.0	50.2	75.2	<0.001
2001–2010	8.2	34.8	57.5	<0.001
After 2011	0.9	4.2	8.2	0.570

Table 23 Overall decrease (%) of WHO_{2005}-TEQ in human milk per 1 year, 5 years and 10 years in countries of the Western European and Others Group and for four countries, estimated concentrations (pg WHO_{2005}-TEQ/g lipid) in 2025 (calculated by the Theil-Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years]	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Australia	5.1	22.9	40.6	2.1	0.008
Austria	5.6	25.2	44.0		0.250
Belgium	5.9	26.3	45.7	3.7	<0.001
Finland	7.0	30.3	51.4		<0.001
Germany	7.4	31.8	53.5	3.1	<0.001
Ireland	4.0	18.5	33.6		0.134
Luxembourg	7.8	33.3	55.5		0.500
Netherlands	6.8	29.8	50.7	4.0	0.016
New Zealand	5.6	25.0	43.7		0.250
Norway	7.0	30.3	51.5		<0.001
Spain	6.9	30.1	51.1		<0.001
Sweden	6.4	28.0	48.1		0.25
Switzerland	6.1	26.9	46.6		1.000
Median	6.4	28.0	48.1		

6 Summary and Conclusions

One of the objectives of the WHO/UNEP-coordinated exposure studies in human milk was to generate comparable and consistent monitoring data to estimate time trends on levels of POPs included in the Stockholm Convention on Persistent Organic Pollutants as required under Article 16 of the Convention. The Convention parties adopted the *Guidance Document on the Global Monitoring Plan* that considers necessary data on the levels of these POPs in certain environmental compartments and human tissues (blood and milk) to estimate time trends to measure the effectiveness of the Convention in eliminating or reducing emissions of POPs.

The presentation and discussion of the 2000–2019 results in Part III of this compendium includes a *general* estimation of time trends during the five rounds for *all* participating countries over these 20 years using data gathered from various rounds. However, a more precise approach for determining time trends is the evaluation of results from *only countries with repeated participation* in these studies: This allows more certainty in drawing of conclusions on temporal trends, which are not potentially influenced by single results of a countries submitted for a single round, and seemed optimal for the evaluation of the effectiveness for the purpose of Article 16.

For statistically significant *trends*, a minimum of five data points have to be available. However, for most countries only less than five data points are available. This prevents deriving statistically significant temporal trends in these cases. Yet, the existing data can indicate decreasing or increasing *tendencies* in POP concentrations. Furthermore, pooling of data in regions allows to derive statistically significant time trends for the UN Regional Groups. To minimize possible sources of variation for time trend analysis of POPs, including PCB, PCDD and PCDF, the concept of the WHO/UNEP-coordinated exposure studies has two basic elements (preparation of pooled samples from a number of individual samples considered to be representative for a country or region/subgroup; analysis by a reference laboratory).

This chapter presents temporal trends derived for the (1) sum of six Indicator PCB (**ΣPCB_6**), (2) sum of toxic equivalents (TEQ) of PCDD/PCDF (**WHO-PCDD/PCDF-TEQ**) and (3) total sum of toxic equivalents ("Total TEQ") of mixtures of PCDD/PCDF and dioxin-like PCB (**WHO_{2005}-TEQ**). All concentrations are reported on a lipid basis.

Five rounds of WHO/UNEP-coordinated exposure studies from 2000–2019 as well as two WHO-coordinated exposure studies carried out in 1987–1988 and 1992–1993 are used as the basis of this evaluation. Therefore, data for human milk samples from 57 countries with repeated participation from all United Nations Regional Groups between 1987 and 2019 were available to derive time trends.

Decreases *(decrease rate constants)* per 1 year, 5 years (approximately 20% higher than the decrease over 4 years as average lengths of the WHO/UNEP-coordinated studies) and 10 years were computed using the Theil-Sen trend estimator including values of all individual samples for the trend analysis. For confirmation, a method of deriving the regional trend as a median of trends in countries within the region was used ("median method").

The reduction rates should be seen also in context with the concentration range (differentiation of levels above or in the range of the background contamination). If high levels are found, sources might be detected which could be eliminated. However, at low background levels, other factors, e.g. contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally.

Indicator PCB

In European countries, the sum of 6 non-dioxin-like Indicator PCB was in a range up to 800 ng/g at the end of the 1980s and early 1990s and decreased to mostly below 100 ng/g until 2019. This was approximately the maximum concentration found in non-European regions over the whole period 1987–2019.

A 50% decrease within a 10-year period was achieved for the levels of ΣPCB_6 in nearly all UN regions (range 34% to 65% calculated by the Theil-Sen method; range 45% to 62% calculated by the median method) and at a global level (71% calculated by the Theil-Sen method; 56% calculated by the median method). The Latin American and Caribbean countries had lower ΣPCB_6 concentrations in comparable periods, obviously resulting in lower decreases (*decrease rate constants*). This is an

indication that the decrease might be faster in regions with higher concentration, compared to a slower decrease in less polluted regions.

As an example for European countries, in Germany a decrease of ΣPCB_6 concentrations in human milk by 95% was achieved between the end of the 1980s and 2019. Similarly, Belgium, The Netherlands, the Czech Republic, the Slovak Republic, Austria and Lithuania were successful with reductions in the range of 85% to 95% between the end of the 1980s/early 1990s and the period between 2012 and 2019. Most other European countries also reduced ΣPCB_6 concentrations in human milk substantially, although often over a shorter period. This trend is largely influenced by the stop of PCB production in the 1980s and a restriction of PCB use in open application in the 1970s with improved PCB waste management from 1990s on including Stockholm Convention measures from 2001 on (Weber et al. 2018).

As in European countries, Vietnam and Thailand also saw a decrease of ΣPCB_6 concentrations in human milk by 85% to 95% between the end of the 1980s and 2016–2019, however, starting at considerably lower concentrations in both periods. In Egypt and Fiji, a reduction between approximately 85% and 75% was observed from 2000–2003 to 2016–2019. In Hong Kong SAR of China, ΣPCB_6 concentrations fell approximately 50% from 2000–2003 to 2008–2011, and in Brazil about 60% from 2000–2003 to 2012–2015. Most other countries participated for the first time in the period 2008–2011. In nearly all countries, decreases were observed in the following years but there is not enough data to derive the time trends. In Mexico, the concentration remained unchanged at low background levels. Tendencies for increase were seen in Senegal and the Marshall Islands.

WHO-PCDD/PCDF-TEQ

At the global level, the decrease in the levels of PCDD/PCDF levels within a 10-year period was 48% calculated by the Theil-Sen method and 34% calculated by the median method. A decrease over 10 years of 51% was achieved in the Western European and Others Group with initially relatively high WHO-PCDD/PCDF-TEQ concentrations in the first (1987–1988) and second (1992–1993) rounds. Most Eastern European countries participated for the first time in the 1992–1993 period with on average lower WHO-PCDD/PCDF-TEQ concentrations in human milk than in Western European countries; for this group, the decrease within a 10-year-period was 35%.

In comparison with the Western European and Others Group and the Eastern European Group, a higher variation of decreases (*decrease rate constants*) between countries was observed in other UN Regional Groups. Lower decreases were observed in some countries, but this has to be seen in context with the quite low levels in these countries.

Overall in 7 periods between 1987 and 2019, the median of concentrations of WHO-PCDD/PCDF-TEQ decreased considerably from 16.9 pg/g in 1987–1988 as averaged for 13 countries to 2.68 pg/g in 2016–2019 as averaged for 33 countries, which is a reduction of 84%. Because the Stockholm Convention was adopted in 2001 and entered into force in 2004, the levels from period 2000–2019 may be taken

at the base. In this case, the initial concentration would be 7.34 pg/g in the period 2000–2003, which still shows a significant reduction of 63% until 2016–2019.

As an example for European countries, the Netherlands, Belgium and Germany had the highest WHO-PCDD/PCDF-TEQ concentrations in the period 1987–1988 (ranging from 32–42 pg/g). As a result of numerous measures taken already in the 1990s to detect and eliminate dioxin sources, a substantial decline of about 90% was observed in human milk to 4.48 pg/g found in the Netherlands in 2014, 4.03 pg/g found in Belgium in 2015 and 2.90 pg/g found in Germany in 2019. Belgium is the country with the highest number of participations ($n = 6$); its results show that the most substantial decrease occurred between 1987–1988 and 2004–2007 with slight decreases afterwards.

Two Asian countries also participated in the 1987–1988 round. Significant decreases in WHO-PCDD/PCDF-TEQ concentration were observed in Vietnam (by 86% until 2019) and Thailand (by 67% until 2018). Increasing concentrations were found in the Marshall Islands. In all other countries the concentrations decreased by smaller amounts or remained stable in the range of levels of background contamination.

Total TEQ (WHO_{2005}-TEQ)

As dioxin-like PCB were of interest starting from the second WHO round (1992–1993), time trends for total TEQ (WHO_{2005}-TEQ) can be derived only from this period. Similar temporal trends as described for WHO-PCDD/PCDF-TEQ were observed. Discrepancies depend on dioxin-like PCB and their contribution to total TEQ in comparison with the PCDD/PCDF contribution.

In nearly all countries, WHO_{2005}-TEQ concentrations were consistently decreasing over the whole monitored period. A decrease around 50% was achieved in regions with countries with initially relatively high WHO_{2005}-TEQ concentrations in human milk, as found, e.g., in Western Europe in the second (1992–1993) or third (2000–2003) round or in an African country in the 2000–2003 period. Most Eastern European countries had on average lower WHO_{2005}-TEQ concentrations in human milk than Western European countries; for the Eastern European group, the decrease within a 10-year-period was nearly 40%. The lower decreases (*decrease rate constants*) observed in some African, Pacific, Latin American and Caribbean and Eastern European countries are in line with the quite low levels in these countries in comparison with some countries of other regions and their periods of participation.

Overall Conclusions

The concept of WHO/UNEP-coordinated exposure studies with standardized protocols for preparation of pooled samples considered to be representative for a country or subpopulation in a country and analysis in a reference laboratory provides reliable data for human milk samples. The consideration only of countries with repeated participation over a decade or more provides the best possible database assessment of temporal trends. Statistically significant decreasing trends for PCB and PCDD/PCDF were observed for all parameters in the five UN Regional Groups and at a global level. However, for the majority of individual countries the limited

available data did not allow for the statistically significant assessment of time trends, but decreasing tendencies were observed for many of them and constant or increasing levels for few of them. It is highly recommended to continue this monitoring effort to secure enough data for appropriate time trend assessments in the future.

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing Regional Support for the POPs Global Monitoring Plan Under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, is greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratory, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the Reference Laboratory was an important aspect for the successful organization of these studies on a global level.

Data analysis was supported by the RECETOX Research Infrastructure (No LM2018121) and the CETOCOEN EXCELLENCE Teaming Phase 2 project financed by the European Union's Horizon 2020 (No 857560) and the European Structural and Investment Funds: No CZ.02.1.01/0.0/0.0/17_043/0009632).

Hae Jung Yoon, Seongsoo Park and Philippe Verger (Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

We thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

We also thank the team at CVUA Freiburg for performance of the analyses and for the scientific support and cooperation with the national coordinators.

Disclaimer The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions, policy or views of the World Health Organization and the United Nations Environment Programme.

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Time Trends in Human Milk Derived from WHO- and UNEP-Coordinated Exposure Studies, Chapter 2: DDT, Beta-HCH and HCB

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Abstract

Temporal trends of DDT ("DDT complex" as sum of p,p′-DDT, o,p′-DDT, p,p′-DDD and p,p′-DDE), beta-hexachlorocyclohexane (beta-HCH) and hexachlorobenzene (HCB) were assessed using pooled human milk samples from 44 countries from all United Nations Regional Groups based on their repeated participation in WHO/UNEP-coordinated exposure studies performed between 2000 and 2019. In contrast to a general estimation of time trends based on results from all countries, this is a more precise approach, because levels among countries are often highly variable. The primary objective of these temporal studies is to provide monitoring data for the effectiveness evaluation of the Stockholm Convention on Persistent Organic Pollutants (POPs).

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_13

For DDT, an overall decrease over 10 years between 50% and 80% was achieved in Africa, the Asia-Pacific region and in Latin America and the Caribbean region, and at a global level. Slightly lower decreases were observed in European countries because DDT was banned much earlier in these countries and only residual levels were depleting. Western European countries had the lowest median and the lowest maximum DDT concentrations. This is an indication that the decrease might be faster in regions with higher concentrations, compared to a slower decrease in less contaminated regions. The frequency distribution of the country-specific decrease (*decrease rate constants*) confirms these findings.

For beta-HCH, an overall decrease over 10 years between 50% and 98% was achieved in all UN regions and at a global level. Country-specific decreases vary in the low background range (below 5 µg beta-HCH/kg lipid). Regarding HCB, all countries from Africa and many countries from the Pacific Islands and Latin America and the Caribbean were in the range of a low background contamination below 5 µg/kg lipid resulting in a wide range of reduction rates. In contrast, in countries with HCB concentrations above 30 µg/kg lipid in previous rounds, overall decreases over 10 years between 50% and 85% were observed.

Therefore, the reduction rates should be seen also in context with the concentration range: A differentiation of levels above or in the range of the background contamination seems to be advised. If high levels are found, sources might be detected which could be eliminated. However, at low background levels, other factors, e.g. contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally.

Keywords

Time trends · Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · DDT · Beta-hexachlorocyclohexane (beta-HCH) · Hexachlorobenzene (HCB) · Global WHO/UNEP studies · UN Regional Groups

1 Introduction

The World Health Organization (WHO) and the United Nations Environment Programme (UNEP) performed between 1987 and 2019 seven exposure studies on human milk. The assessment of temporal trends is a key element for the effectiveness evaluation of the Stockholm Convention on Persistent Organic Pollutants (POPs) and human milk is a core matrix for this purpose. In this compendium, specific papers address various aspects of these human milk surveys, including in Part I a review of human milk surveys on POPs from a historical perspective (Fürst 2023), the overview of the WHO/UNEP-coordinated exposure studies (Malisch et al. 2023a) and a review of the Stockholm Convention, the Global Monitoring Plan (GMP) and its implementation in regional and global monitoring reports (Šebková 2023).

Worldwide trends in DDT concentrations in human breast milk were assessed compiling data since 1951 until the end of the 1990s (Smith 1999). A global

overview on the spatial and temporal trends of Stockholm Convention POPs in breast milk reviews scientific publications between 1995 and 2011 (Fång et al. 2015). The regional and global monitoring reports for the GMP assess datasets in the core media—ambient air, human tissues (human breast milk or blood) and water for hydrophilic POPs, but also other media such as soil, biota, plants are used to support interpretation of observed levels and their trends. These reports are available at the homepage of the Stockholm Convention (>Implementation>Global Monitoring Plan>Monitoring Reports).

In three articles of Part IV of this compendium, time trends derived from the WHO/UNEP-coordinated exposure studies are evaluated, in the first article ("Time Trends in Human Milk Derived from WHO- and UNEP-coordinated Exposure Studies, Chapter 1") for polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (Malisch et al. 2023b). This includes a clarification of quantitative goals of temporal studies and Convention's objectives: To provide reliable monitoring information for the Parties to the Stockholm Convention, as a quantitative objective for temporal studies the GMP guidance document proposed the ability to detect a 50% decrease in the levels of POPs within a 10-year period. However, there is no stipulation of a quantitative goal for the rate of reduction/decrease in POPs levels. The Convention's objectives are either to eliminate or to reduce production, use and releases, depending on the annex where a chemical is listed, but the rate of decline is nowhere specified or required (UNEP 2015, 2019).

For chlorinated pesticides and industrial chemicals, the presentation and discussion of the 2000–2019 results in Part III includes a first *general* estimation of time trends in the five rounds for all countries participating over these 20 years (Malisch et al. 2023c). With regard to the concentrations found, most relevant were **DDT** (expressed as "DDT complex" and comprised of p,p′- and o,p′-isomers of DDT [dichlorodiphenyltrichloroethane] and the transformation products p,p′-DDE [dichlorodiphenyldichloroethylene] and p,p′-DDD [dichlorodiphenyl dichloroethane]), beta-hexachlorocyclohexane (**beta-HCH**) and hexachlorobenzene (**HCB**). As chapter 2 of the three articles on time trends, this paper presents the results of a more precise approach for the assessment of these three POPs by consideration of *results only from countries with repeated participation* in the studies.

2 General Aspects

2.1 Minimization of Sources of Variation; Samples and UN Regional Groups; Long-Term Quality Control

For minimization of possible sources of variation from the sampling design, in all rounds a number of individual samples were collected and pooled (mixed) samples prepared following a standardized protocol that was supervised by national coordinators. Equal aliquots of individual samples were combined to give composite

samples, which are considered representative of the average levels of the analytes of interest in human milk for a certain country or subpopulation of a country at the time of sampling. For an overview of the scope, protocols for collection of samples, factors minimizing sources of variation for assessments of time trends, participation of countries and the five UN Regional Groups (African Group; Asia-Pacific Group; Group of Latin American and Caribbean Countries [GRULAC]; Eastern European Group; Western European and Others Group [WEOG]), see (Malisch et al. 2023a), for results of organochlorine pesticides determined in 163 pooled human milk samples from 82 countries, see (Malisch et al. 2023c).

For minimization of the variation from chemical analysis at the 2000–2019 WHO/UNEP-coordinated studies, the determination of organochlorine pesticides and industrial contaminants in the pooled samples was performed by the Reference Laboratory applying long-term analytical quality control (Hardebusch et al. 2023).

Due to the particular scope at the beginning of a study with regard to the expansion of analytes of interest over time, results from 119 pooled samples from 44 countries with repeated participation between 2000 and 2019 are available for various organochlorine pesticides and industrial chemicals, including DDT complex, beta-HCH and HCB as covered in this article. The detailed data for all samples is contained at the POPs Global Monitoring Plan Data Warehouse (GMP DWH) and can be publicly retrieved (GMP DWH 2020).

2.2 "DDT Complex" as Sum Parameter for DDT

The sum parameter "DDT complex" is calculated as sum of o,p′-DDT, p,p′-DDT, p,p′-DDE and p,p′-DDD using correction factors for molecular weight for the metabolites p,p′-DDE and p,p′-DDD. This term is used in combination with the unit to express concentrations; alternatively, the common short form "DDT" is used. The extent of the contribution of p,p′-DDT and p,p′-DDE to DDT complex is indicative of an older or more recent use of DDT (Malisch et al. 2023c).

2.3 Methods of Statistical Data Treatment: Trends vs. Tendencies

If a country had sent two or more pooled samples in a certain round, the median of these samples in this period has been used for the country in some summarizing figures and tables. These are identified as "country results" or "aggregated data". However, for the time trend analysis, data were not aggregated, but values of all individual pooled samples were used.

For methods of statistical data treatment (use of the non-parametric linear Theil–Sen trend estimator and of the median method to derive decrease rates expecting exponential trends [as commonly observed in cases after stop of production and application of a chemical rather than unrealistic linear trends] and for prevention of Simpson's paradox), see subsection 2.4 in the preceding chapter "Time Trends in Human Milk Derived from WHO- and UNEP-coordinated Exposure Studies,

Chapter 1" on time trends for PCB, PCDD and PCDF (Malisch et al. 2023b). This includes the differentiation between *trends* as statistically significant decreases (*decrease rate constants)* requiring *p*-values <0.05 and changes of concentrations indicating *tendencies* as statistically not significant decreases. As Theil–Sen p is never below 0.05 for less than 5 data points and for most countries only less than 5 data points were available, statistically significant trends could be derived only for regions (combining data from countries) and few countries, showing on 95% confidence level whether the trend is not caused by random variance in the data. In addition, for some countries, based on statistically significant reduction rates and participation also in the decade after 2010, a prognosis of the estimated concentrations in 2025 was derived. However, for most countries, only two or three data points are available. In these cases, the observed changes of the concentrations do not allow to draw statistically significant conclusions on trends and therefore indicate tendencies.

2.4 Background Concentration

As explained in the chapter on findings of organochlorine pesticides and industrial chemicals (Malisch et al. 2023c), a background concentration is defined as that portion of the measured human milk levels that is found in the absence of specific sources, e.g., the chemical of interest was not used or emitted within the study area, or after a sufficiently long phase-out period (depending on the half-life). In contrast to findings of high concentrations, e.g. after use of chemicals, the low levels described as "background levels" are not attributable to a known emission source.

However, the term "background level" does not imply per se any level of safety. With respect to potential adverse effects, risk assessments need to consider many factors, including the toxicity of the chemical of interest and the measured concentration range. For human milk, potential adverse effects have to be balanced against its many known positive health aspects for breastfed infants.

3 DDT

3.1 Global level and comparison between UN Regional Groups

For DDT concentrations in human milk, large differences were found among the 119 pooled samples from 44 countries with repeated participation between 2000 and 2019: The range between a minimum of 17 μg DDT complex/kg lipid found in 2019 and a maximum of 23,500 μg DDT complex/kg lipid found in 2012 covered three orders of magnitude (median: 283 μg DDT complex/kg lipid). This is the same range as found in 134 country results (based on aggregated data) of 82 countries (as total number including one-time participants between 2000 and 2019), with a median of 255 μg DDT complex/kg lipid (Malisch et al. 2023c).

As recommended by the GMP guidance document (UNEP 2019), the Theil–Sen method was applied for power analysis of statistical trends. Statistically significant trends were derived for the UN Regional Groups by combination of data from countries. Basic results of the exponential trends calculated by this method comprise the overall decreases per 1 year and 10 years. Statistical differences between the Theil–Sen method and the additionally applied median method to derive time trends in different UNEP Regional Groups were insignificant on 95% confidence level, which shows that the Simpson's paradox caused by different sampling periods is weak in these cases (Table 1). For the country-specific results in the following subsections 3.2–3.6, also decrease rates per 5 years are shown, which are about 20% higher than the decrease rates per 4 years representing the average lengths of WHO/UNEP-coordinated exposure studies.

An overall decrease within a 10-year period between 50% and 80% was achieved for DDT levels in Africa, the Asia-Pacific Group and the Group of Latin American and Caribbean Countries, and at a global level. Lower reduction rates were observed in the Eastern European Group and the Western European and Others Group, which had banned DDT much earlier. Generally, the highest DDT concentrations in the five periods between 2000 and 2019 were found either in Africa or in the Asia-Pacific Group or in the Group Latin America and Caribbean Countries, whereas Western European and Others Group countries had the lowest median and lowest maximum of DDT concentrations. This is an indication that the decrease might be faster in regions with higher initial concentrations, compared to a slower decrease in less contaminated regions. All trends were statistically significant (p-value <0.001 and 0.017, respectively).

On a global level, the decrease over 10 years was nearly 60% calculated by the Theil–Sen method with use of all individual samples. The median method

Table 1 Overall decrease (%) of DDT concentrations (calculated as DDT complex) in human milk in the five UN Regional Groups and worldwide (computed using all samples from countries with repeated participation; for UN Regional Groups, in particular for "Others" in the "Western European and Others" UN Regional Group, see Malisch et al., 2023a and 2023c and subsection 3.6)

UN Regional Groups	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall[a]
		Theil–Sen method	Median method	Theil–Sen method	Median method	
Africa	13	12.7	14.5	74.3	79.1	<0.001
Asia-Pacific	8	15.0	10.3	80.2	66.5	<0.001
Latin America and Caribbean	9	6.6	7.7	49.5	55.0	0.017
Eastern Europe	6	4.7	5.9	38.5	45.5	0.017
Western Europe and Others	8	4.8	6.4	39.1	48.6	<0.001
Global	44	8.4	6.6	58.3	49.5	<0.001

[a]For Theil–Sen method

(as median of the 5 UN Regional Groups and of the 44 countries) gave comparable results.

The exponential trends of DDT concentrations in human milk derived by the Theil–Sen method in the five UN regions and worldwide by combination of data from countries are illustrated in Fig. 1a–f. These figures are normalized according to the maximum concentration found in the respective UN Regional Groups. Thus, the different scales illustrate the different ranges between and within the UN Regional Groups. For a detailed discussion of the regional data, see the following subsections 3.2–3.6.

3.2 African Group

Figure 2 (for aggregated data, see subsection 2.3) and Fig. 3 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate temporal changes of DDT concentrations in 13 countries from Africa with repeated participation between 2000 and 2019. In Egypt, a reduction of 96% was observed from nearly 400 μg DDT complex/kg lipid in the 2000–2003 period to the 2016–2019 period, when with 17 μg DDT complex/kg lipid the lowest DDT concentration of all countries on a global level during the whole period 2000–2019 was found. Most countries participated for the first time in the 2008–2011 period; in these countries, DDT concentrations fell on average by about 70% until the period 2016–2019 (range between 59% and 93% decrease). The highest concentration of 23,500 μg DDT complex/kg lipid (or 23.5 mg/kg) found in Ethiopia in 2012 decreased by 70% until 2019. For discussion of use of DDT to combat mosquitos for malaria control and measures taken by Ethiopia to successfully reduce DDT levels, see (Gebremichael et al. 2013; Malisch et al. 2023c).

The overall decreases per 1 year, 5 years and 10 years are given in Table 2. The limited number of samples did not allow to determine statistically significant decrease rates ($p \sim 1.000$) (for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.3). On average, the levels of DDT in all African countries decreased within a 10-year period by nearly 80% (median 79%; range 63–95%). This is in line with the statistically significant ($p < 0.001$) decrease over 10 years for all African countries of 74% calculated by the Theil–Sen method (see Table 1 in Sect. 3.1).

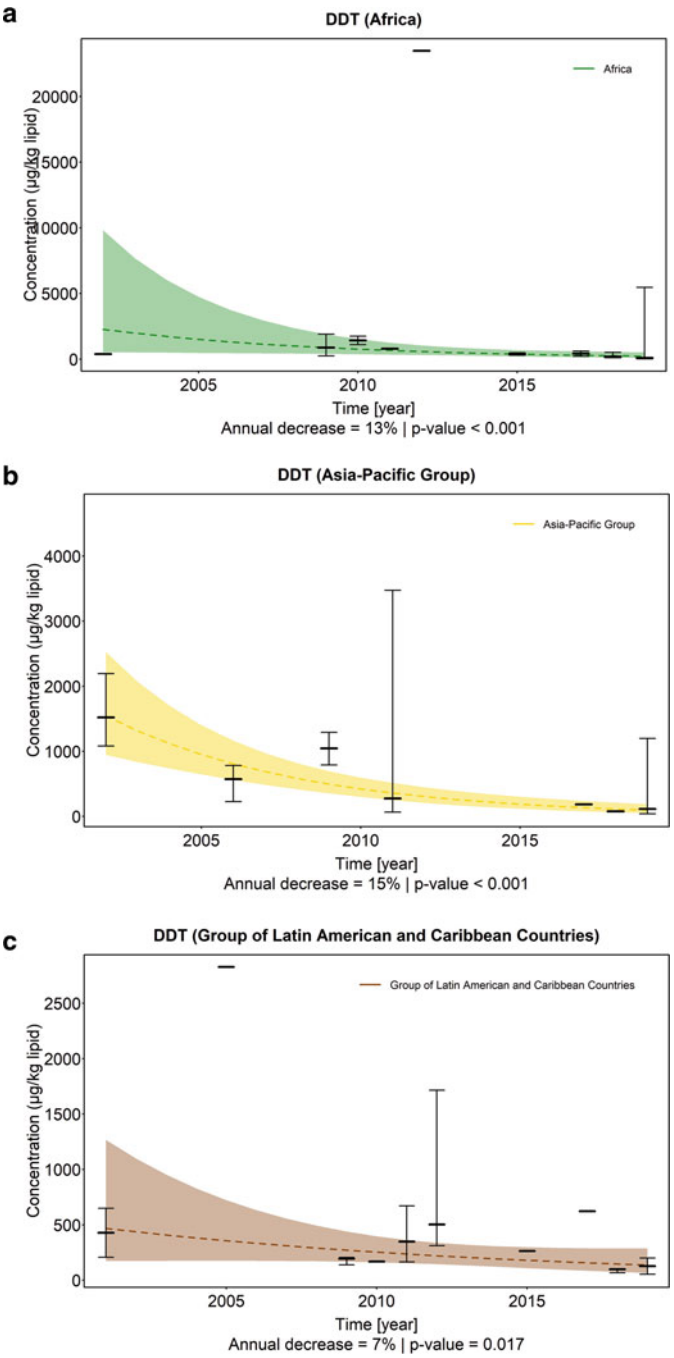

Fig. 1 (**a–f**) Theil–Sen exponential trends of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid) worldwide and in the five UN regions. The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, whiskers show ranges between fifth and 95th percentiles

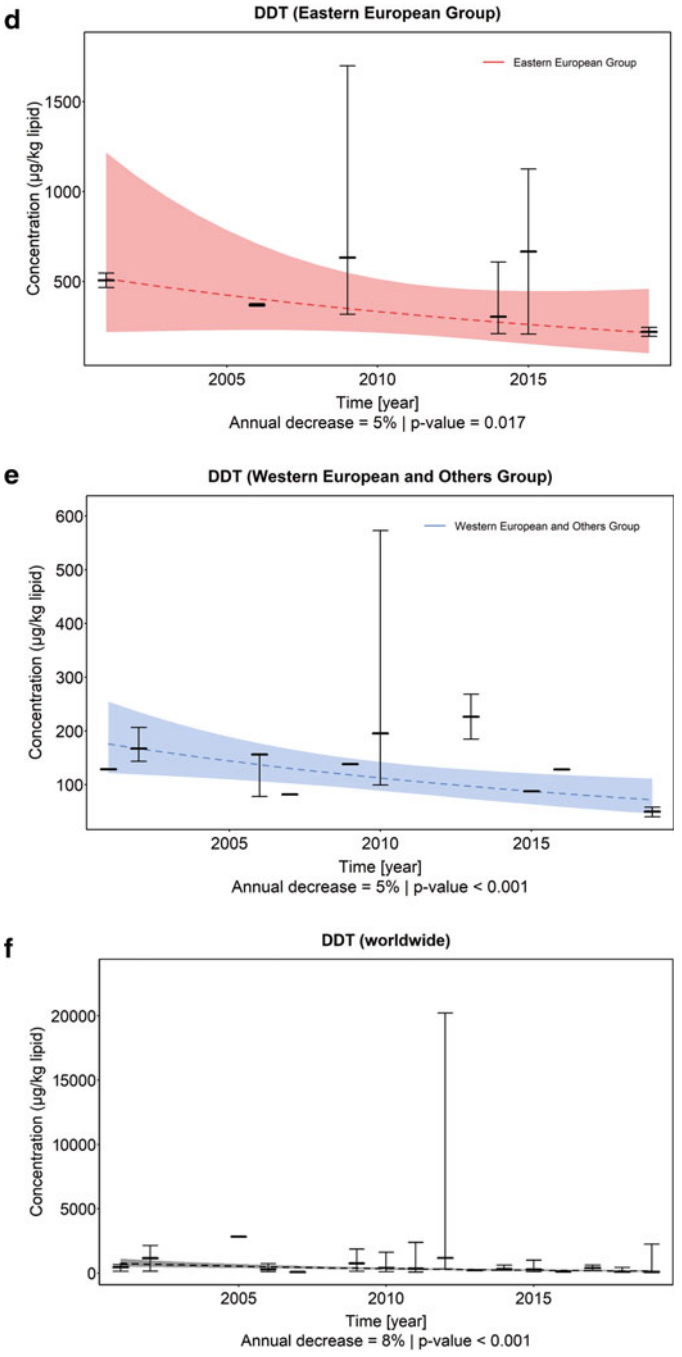

Fig. 1 (continued)

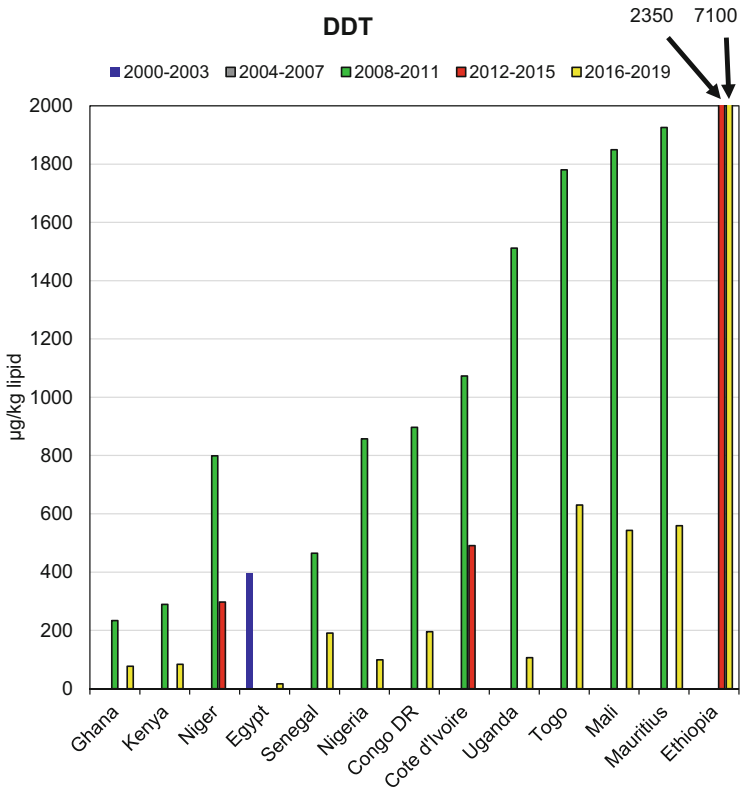

Fig. 2 Overview of the development of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid; aggregated data) over time in African countries with repeated participation between 2000 and 2019

3.3 Asia-Pacific Group

Figures 4 (for aggregated data) and 5 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of DDT concentrations in 8 countries from the Asia-Pacific Group with repeated participation between 2000 and 2019.

In Hong Kong SAR of China, DDT concentrations fell approximately by 35% from the 2002 level of 1580 µg DDT complex/kg lipid (median of 10 samples from different population subgroups, see Hui et al. 2008) to the 2009 level of 1040 µg DDT complex/kg lipid (median of 4 samples from different subgroups). In Fiji, a reduction of nearly 80% was observed from 2000–2003 to 2008–2011, which then was further reduced by about 60% until 2016–2019 to 8% of the initially found concentration (1340 µg DDT complex/kg lipid in 2001). Most other countries

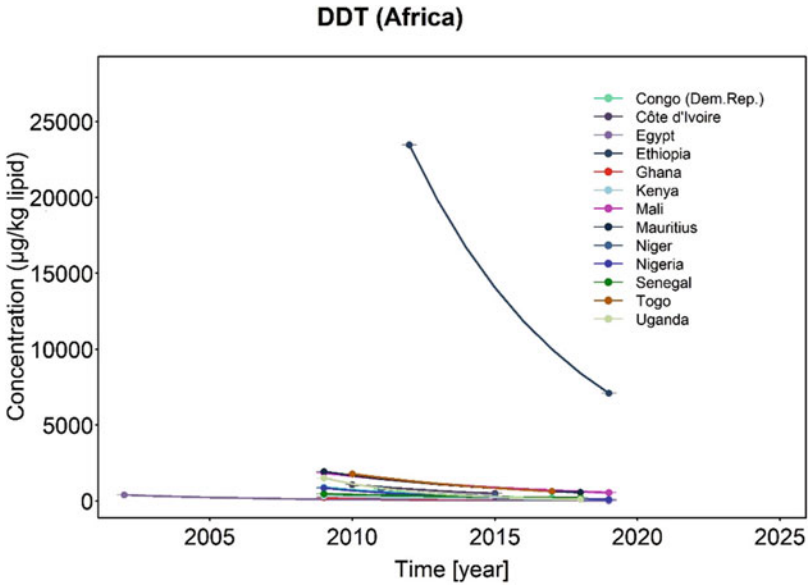

Fig. 3 Temporal tendencies of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid) in African countries with repeated participation between 2000 and 2019 using the Theil–Sen method

Table 2 Overall decrease (%) of DDT concentrations (expressed as µg DDT complex/kg lipid) in human milk in African countries per 1 year, 5 years and 10 years (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Trend *p*-value overall
Congo (DR)	17.3	61.4	85.1	1.000
Côte d'Ivoire	14.5	54.3	79.1	1.000
Egypt	17.0	60.6	84.5	1.000
Ethiopia	15.7	57.4	81.9	1.000
Ghana	10.5	42.7	67.1	1.000
Kenya	11.7	46.2	71.0	1.000
Mali	11.5	45.8	70.6	1.000
Mauritius	12.8	49.7	74.7	1.000
Niger	21.9	71.0	91.6	1.000
Nigeria	19.4	66.0	88.5	1.000
Senegal	9.4	39.0	62.8	1.000
Togo	13.8	52.4	77.3	1.000
Uganda	25.5	77.1	94.7	1.000
Median	14.5	54.3	79.1	

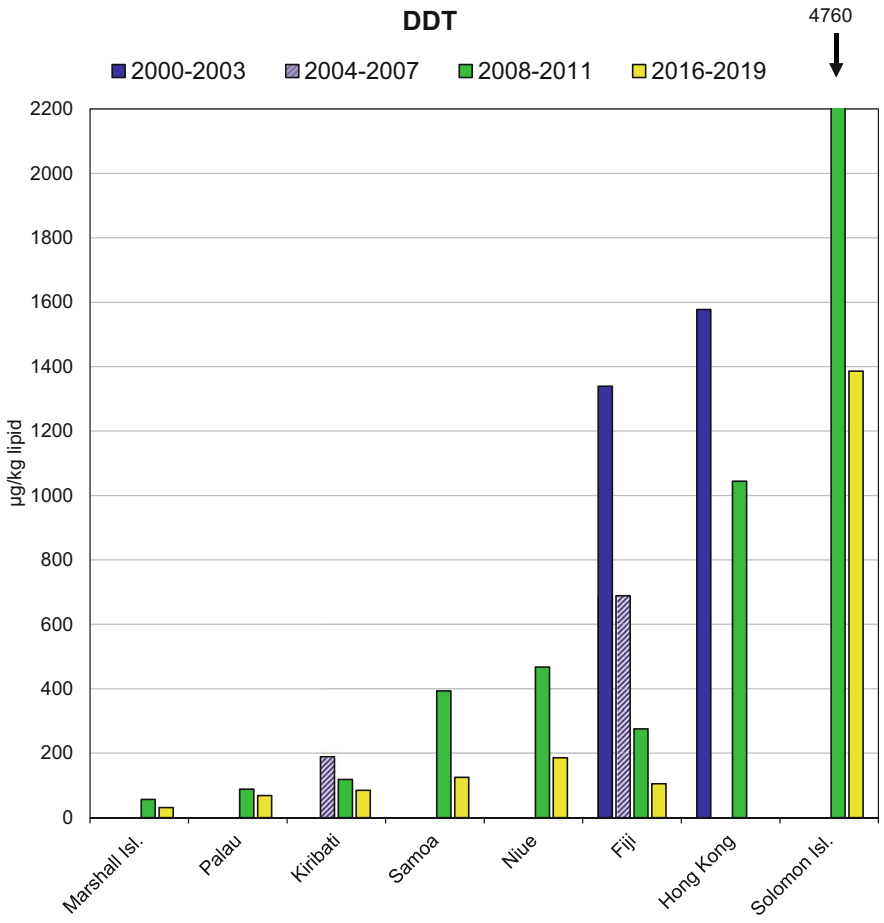

Fig. 4 Overview of the development of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid; aggregated data) over time in countries of the Asia-Pacific Group with repeated participation between 2000 and 2019

participated the first time in the period 2008–2011; Kiribati in 2004–2007. In all these countries, a decrease was observed in the following years (median of decrease: 60%, range 22–71%), including Solomon Islands with a decrease of the high DDT level in 2011 (4760 µg DDT complex/kg lipid) by 71% until 2019.

The overall decreases per 1 year, 5 years and 10 years are given in Table 3. The median of reduction rates in the DDT levels within a 10-year period in 8 countries was 65% (range 31–79%). Statistically significant was the decrease of 47% over 10 years in Hong Kong SAR of China and of 79% in Fiji ($p < 0.001$). The limited

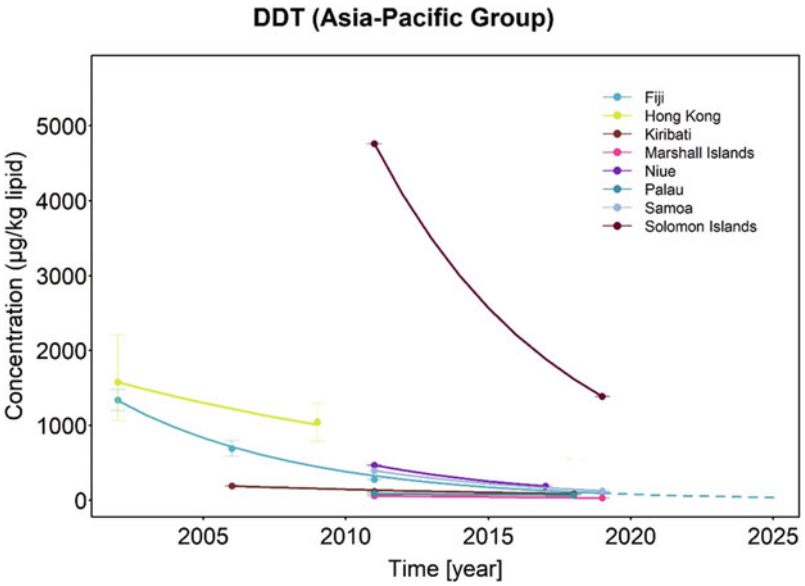

Fig. 5 Temporal tendencies of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid) in countries of the Asia-Pacific Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with statistically significant time trends in Hong Kong SAR of China and Fiji)

Table 3 Overall decrease (%) of DDT concentrations in human milk in countries of the Asia-Pacific Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [µg/kg lipid]	Trend *p*-value overall
Fiji	14.5	54.2	79.0	36.6	<0.001
Hong Kong	6.2	27.4	47.3		<0.001
Kiribati	6.5	28.5	48.8		0.250
Marshall Isl.	7.2	31.3	52.8		1.000
Niue	14.2	53.6	78.5		1.000
Palau	3.6	16.6	30.5		1.000
Samoa	13.4	51.2	76.2		1.000
Solomon Isl.	14.3	53.8	78.6		1.000
Median	10.3	42.1	66.5		

number of samples did not allow to determine statistically significant decreases in the other countries ($p = 0.250$ and 1.000, respectively). For Fiji with a statistically significant decrease and participation also in the 2016–2019 round, in addition a prognosis about the probable concentrations in 2025 was made.

3.4 Group of Latin American and Caribbean Countries (GRULAC)

Figures 6 (for aggregated data) and 7 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of DDT concentrations in 9 Latin American and Caribbean countries with repeated participation between 2000 and 2019.

Based on comparison of median concentrations, in Brazil an increase by 17% was found from 2001 to 2012. In Haiti, DDT concentrations in human milk fell by 91% from 2004 (with 2830 µg DDT complex/kg lipid the highest concentration found in this UN Regional Group) to 2015. Most other countries participated for the first time in the period 2008–2011. In all these countries, a decrease was observed in the following years (median: 41%; range 11–67%).

The overall decreases per 1 year, 5 years and 10 years are given in Table 4. By most countries, a (statistically not significant) decrease in the DDT levels within a 10-year period was achieved (median for 8 countries with reduction rates per 10 years: 60%; range 17–91%). The (statistically not significant) increase calculated

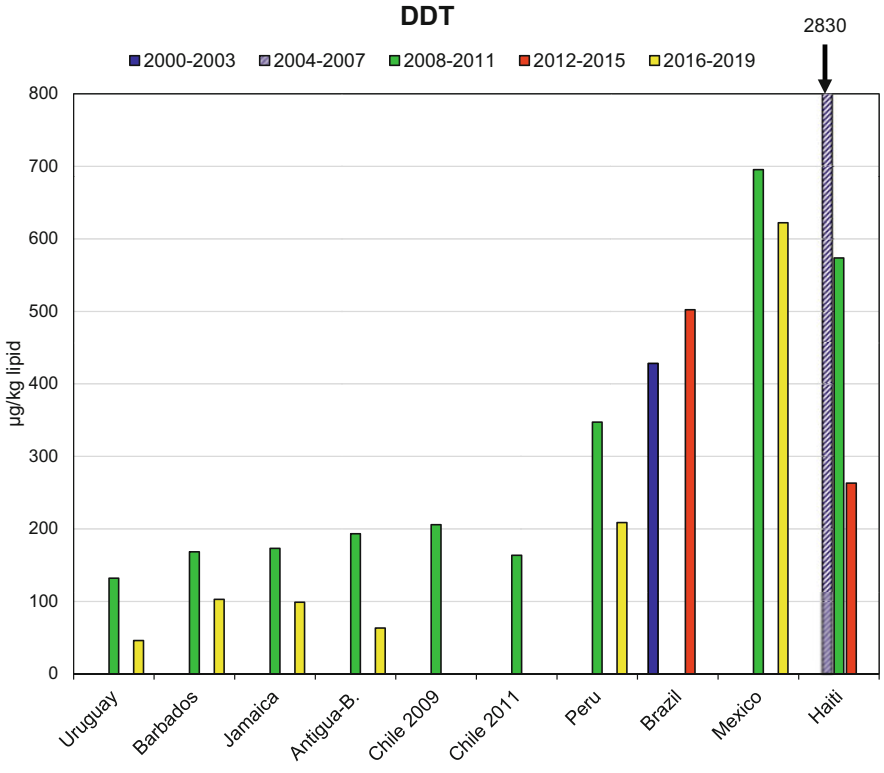

Fig. 6 Overview of the development of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid; aggregated data) over time in Latin American and Caribbean Countries with repeated participation between 2000 and 2019

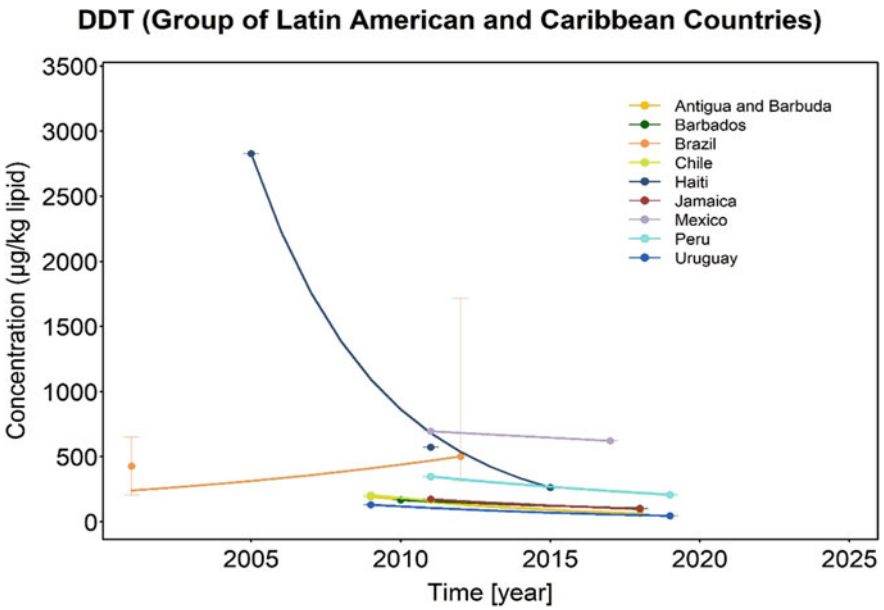

Fig. 7 Temporal tendencies of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid) in Latin American and Caribbean Countries with repeated participation between 2000 and 2019 using the Theil–Sen method

Table 4 Overall decrease (%) of DDT concentrations in human milk in Latin American and Caribbean Countries per 1 year, 5 years and 10 years (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Trend *p*-value overall
Antigua-Barb	11.7	46.3	71.2	1.000
Barbados	6.0	26.5	46.0	1.000
Brazil	−6.9	−39.9	−95.8	0.220
Chile	10.8	43.6	68.2	1.000
Haiti	21.1	69.5	90.7	0.250
Jamaica	7.7	32.9	55.0	1.000
Mexico	1.8	8.8	16.9	1.000
Peru	6.2	27.3	47.1	1.000
Uruguay	10.0	40.9	65.1	1.000
Median	7.7	32.9	55.0	

for Brazil by the Theil–Sen method reflects the high variation within the two sampling periods (182 µg DDT complex/kg lipid and 675 µg DDT complex/kg lipid for the two analysed pooled samples from 2001; 291 µg DDT complex/kg lipid,

502 µg DDT complex/kg lipid and 1850 µg DDT complex/kg lipid for the three national pools from 2012). The median of the decreases (*decrease rate constants*) per 10 years of 55% for all countries is in line with the statistically significant decrease over 10 years for all GRULAC countries of 50% calculated by the Theil–Sen method (see Sect. 3.1).

3.5 Eastern European Group

Figures 8 (for aggregated data, see subsection 2.3) and 9 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of DDT concentrations in 6 countries of the Eastern European Group with repeated participation between 2000 and 2019. Overall decrease rates per 1 year, 5 years and 10 years are given in Table 5.

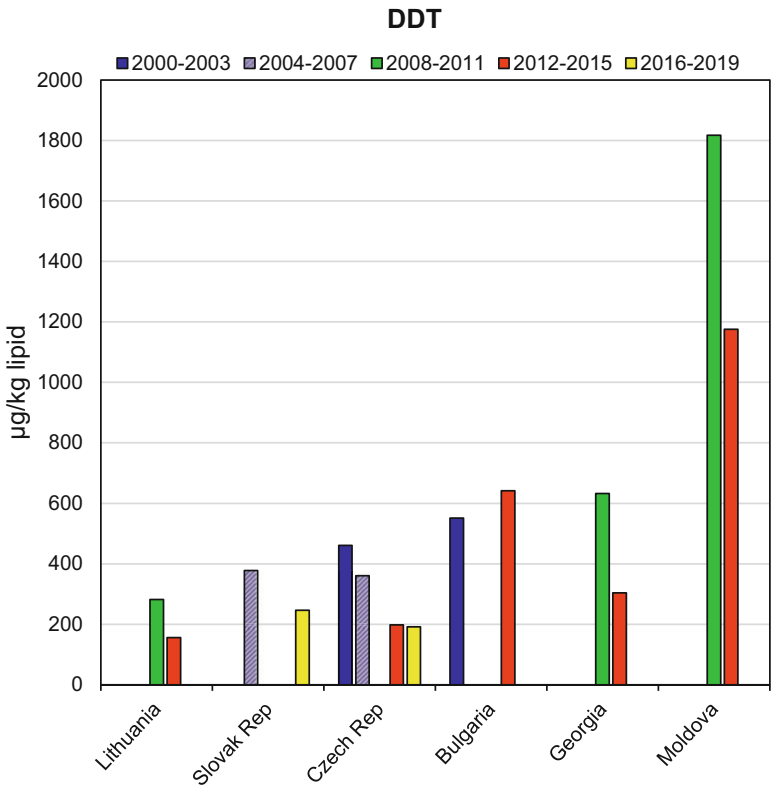

Fig. 8 Overview of the development of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid; aggregated data) over time in countries of the Eastern European Group with repeated participation between 2000 and 2019

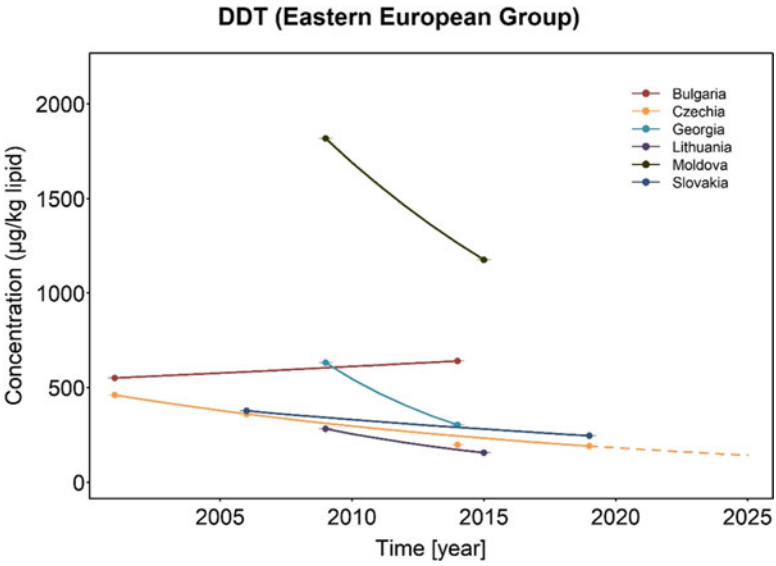

Fig. 9 Temporal tendencies of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid) in countries of the Eastern European Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Czechia)

Table 5 Overall decrease (%) of DDT concentrations in human milk in countries of the Eastern European Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [µg/kg lipid]	Trend *p*-value overall
Bulgaria	−1.2	−6.0	−12.5		1.000
Czechia	4.8	21.7	38.6	143	0.031
Georgia	13.6	51.9	76.9		1.000
Lithuania	9.4	38.9	62.7		1.000
Moldova	7.0	30.4	51.6		1.000
Slovakia	3.2	15.2	28.0		1.000
Median	5.9	26.2	45.5		

A continuous decrease is observed in nearly all countries over all periods. Most data points are available for Czechia (Czech Republic) with four participations between the 2001 and 2019. Here, DDT concentrations in human milk fell from initially 461 µg DDT complex/kg lipid in 2001 by 57% until 2012 and then levelled out remaining quite constant until 2019. The highest concentration found in Moldova in 2009 (1820 µg DDT complex/kg lipid) decreased by 35% until 2015. In Bulgaria, DDT levels increased by 16% from 2001 to 2014.

The median of the reduction rates in the DDT levels within a 10-year period was 45%, with a wide range between countries. In Bulgaria, a slight increase was found,

which was statistically not significant. Statistically significant was the decrease over 10 years of 39% in Czechia; for this country, also a prognosis of the estimated DDT concentration in 2025 was calculated. For the other Eastern European countries, the (statistically not significant) decrease over 10 years was between 28% and 77%.

3.6 Western European and Others Group (WEOG)

Figures 10 (for aggregated data) and 11 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of DDT concentrations in 8 countries of the Western European and Others Group with repeated participation between 2000 and 2019. Western European countries had in comparison to other UN regions quite low DDT concentrations, probably due to early bans on the use of DDT in agriculture implemented in most of these countries. A continuous decrease is observed in the WEOG region nearly over all periods. In Germany, which covers the whole period between 2000 and 2019, a

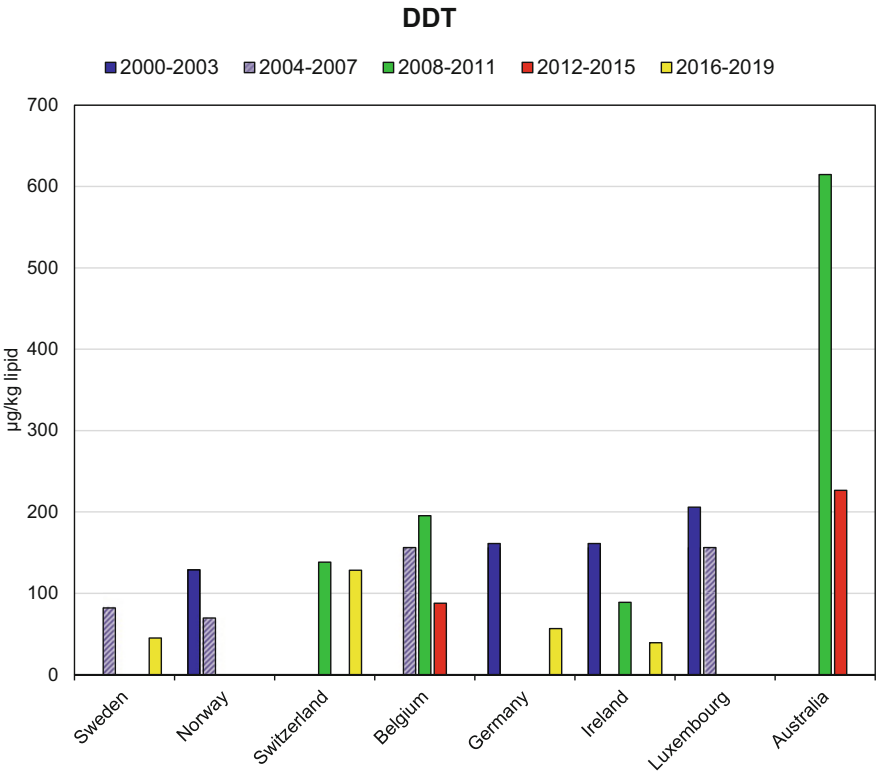

Fig. 10 Overview of the development of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid; aggregated data) over time in countries of the Western European and Others Group with repeated participation between 2000 and 2019

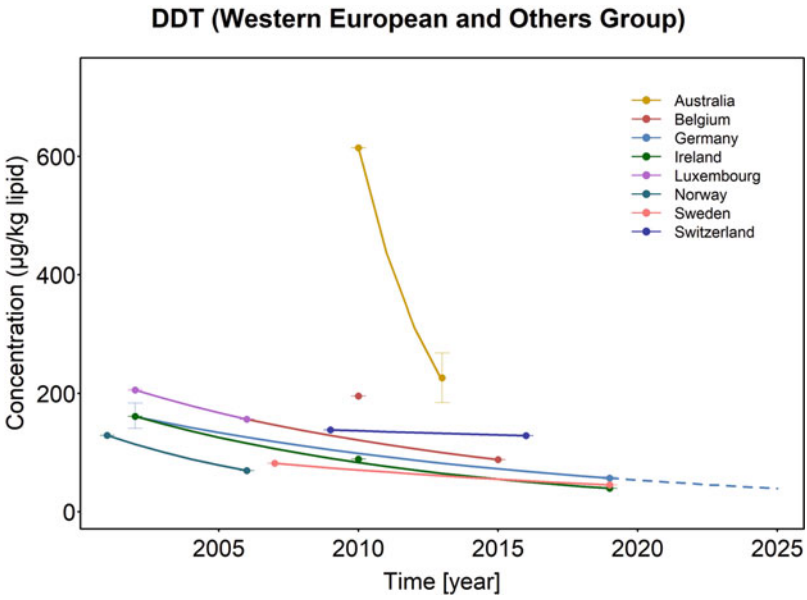

Fig. 11 Temporal tendencies of DDT concentrations in human milk (expressed as µg DDT complex/kg lipid) in countries of the Western European and Others Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Germany)

decrease of DDT concentrations in human milk from 161 µg DDT complex/kg in 2002 (as median of 4 samples) by 65% to 2019 was achieved, with a statistically significant decrease rate over 10 years of 46%. Based on this, also a prognosis of the estimated concentrations in 2025 was derived for Germany (Table 6).

Table 6 Overall decrease (%) of DDT concentrations in human milk in countries of the Western European and Others Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [µg/kg lipid]	Trend *p*-value overall
Australia	28.8	81.7	96.7		0.5
Belgium	6.2	27.4	47.3		0.5
Germany	5.9	26.4	45.8	39.3	0.008
Ireland	7.9	33.9	56.3		0.25
Luxembourg	6.7	29.2	49.9		0.5
Norway	11.6	46.0	70.8		1.000
Sweden	4.9	22.0	39.2		1.000
Switzerland	1.1	5.2	10.1		1.000
Median	6.4	28.3	48.6		

The median of the (statistically not significant) reduction rates for the other 6 Western European countries per 10 years was 49% (range 10–71%) (Table 6). With 97%, the highest decrease over 10 years was found in Australia, which had the highest DDT concentrations in human milk in the WEOG countries (615 μg DDT complex/kg; 2010). For comparison, p,p′-DDE concentrations of 311 ± 174 ng/g lipid were found in a comprehensive study in Australia in 2002–2003 (Mueller et al. 2008). These findings for temporal tendencies are again an indication that the decrease might be higher in areas with initially elevated concentrations and might get lower and finally level out over time when measures were taken to eliminate sources.

3.7 Dependence of Decrease (Decrease Rate Constants) on Concentration

The decrease (*decrease rate constants*) within a 10-year period have to be seen also in context with the concentration range: A differentiation of levels above or in the range of background contamination seems to be advised. If high levels are found, sources might be detected which could be eliminated. For source control, an understanding of country- and chemical-specific use is necessary. Directly after a ban or other measures taken to reduce discharges, concentrations of pesticides can be expected to decrease relatively fast in environmental samples (UNEP 2007, 2015, 2019). However, at background levels, other factors, e.g. contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally, and some fluctuation over time might be expected when concentrations tend to level out.

DDT concentrations in human milk in the 44 countries with repeated participation between 2000 and 2019 comprise a wide range between 17 μg DDT complex/kg lipid and 23,500 μg DDT complex/kg lipid. This was grouped into four ranges (<200; 200–500; 500–2000; >2000) to check the dependence of the decrease rates on the "initial" (the first measured) concentration in a country. The lower end of the frequency distribution was derived from Western European countries, which had in comparison to other UN regions quite low DDT concentrations (many samples <200 μg DDT complex/kg lipid), probably due to early bans on the use of DDT in agriculture implemented in most of these countries. The upper end is related to a more recent use.

Table 7 shows, that the reduction rates over 10 year in the upper part for all three samples with concentrations above 2000 μg DDT complex/kg lipid (decrease in the range 79–91%) are considerably higher than in the lower part (for 13 samples; range 10–71% for decrease of samples <200 μg DDT complex/kg lipid; range 56–193 μg DDT complex/kg lipid and thus two orders of magnitude above LOQ [0.5 μg/kg lipid]). Figure 12 illustrates this dependence of the decrease over 10 years in 44 countries on the concentration range with repeated participation between 2000 and 2019.

Table 7 Overall decrease (%) of DDT concentrations (expressed as μg DDT complex/kg lipid) in human milk over 10 years calculated by the Theil–Sen method and their dependence on the concentration range. Negative decreases are to be read as increase

	<200 μg/kg lipid	200–500 μg/kg lipid	500–2000 μg/kg lipid	>2000 μg/kg lipid
N	13	13	15	3
min	10	−96	−13	79
median	49	63	77	82
max	71	84	97	91

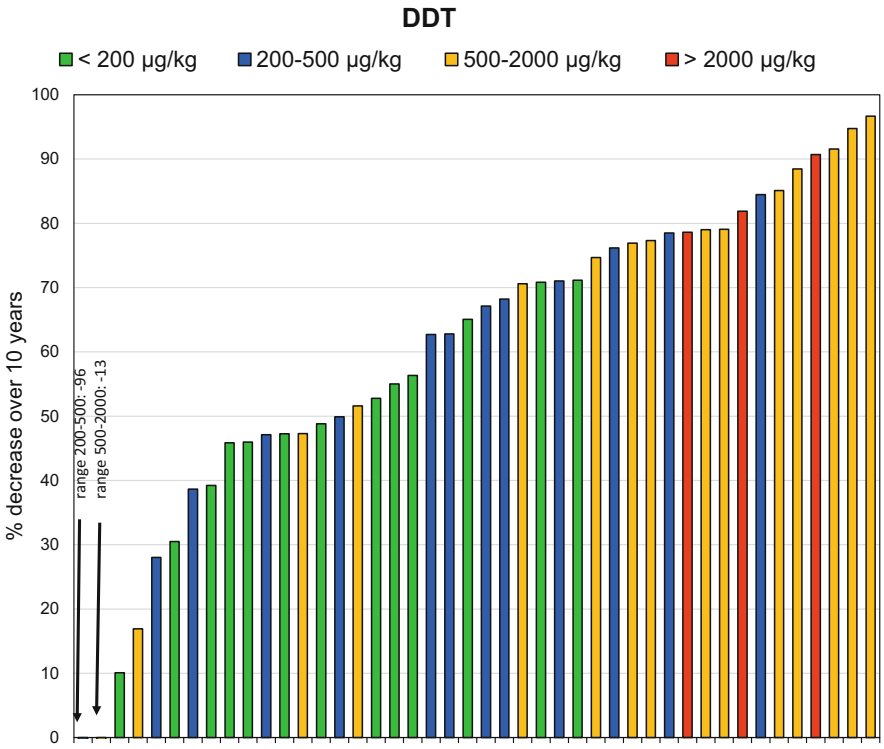

Fig. 12 Dependence of the decrease over 10 years (calculated by the Theil–Sen method) for DDT on concentrations in human milk in 44 countries with repeated participation between 2000 and 2019, with differentiation into four ranges of concentration (<200; 200–500; 500–2000; >2000 μg DDT complex/kg lipid)

4 Beta-Hexachlorocyclohexane (beta-HCH)

4.1 Global Level and Comparison between UN Regional Groups

Large differences of beta-HCH concentrations in 119 pooled human milk samples from 44 countries with repeated participation between 2000 and 2019 were found, with a minimum of <0.5 µg beta-HCH/kg lipid found in few countries and a maximum of 1020 µg beta-HCH/kg lipid found in 2002 (median: 6.0 µg/kg). This is the same range as found in 134 country results (based on aggregated data) of 82 countries (as total number regardless the number of participations between 2000 and 2019), with a median of 5.9 µg beta-HCH/kg lipid (Malisch et al. 2023b).

An overall decrease within a 10-year period between 50% and 98% was achieved for beta-HCH levels in all the UN regions and at a global level. The overall reduction rates per 1 year and 10 years calculated by the Theil–Sen method for exponential trends and the additionally applied median method to derive time trends were quite comparable in nearly all UN regions and at the global level (Table 8). On a global level and in all UN regions except Latin America and the Caribbean, all trends were significant (p-value <0.001 in three regions and globally, 0.037 in Eastern Europe).

The variation of concentration ranges and reduction rates between countries in the five UN Regional Groups is shown in the following subsections.

The exponential trends of beta-HCH concentrations in human milk derived by the Theil–Sen method in the five UN regions and worldwide by combination of data from countries are illustrated in Fig. 13a–f. These figures are normalized according to the maximum concentration found in the respective UN Regional Groups. Thus, the different scales illustrate the different ranges between and within the UN Regional Groups. For a detailed discussion of the regional data, see the following subsections 4.2–4.6.

Table 8 Overall decrease (%) of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in the five UN Regional Groups and worldwide (computed using all samples from countries with repeated participation)

UN Regional Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall[a]
		Theil–Sen method	Median method	Theil–Sen method	Median method	
Africa	13	16.0	14.2	82.5	78.4	<0.001
Asia-Pacific	8	31.0	13.3	97.5	76.1	<0.001
Latin America and Caribbean	9	7.0	6.3	51.9	48.0	0.105
Eastern Europe	6	9.6	7.8	63.4	55.5	0.037
Western Europe and Others	8	9.5	10.2	62.9	65.8	<0.001
Global	44	16.5	9.6	83.5	63.4	<0.001

[a] for Theil–Sen method

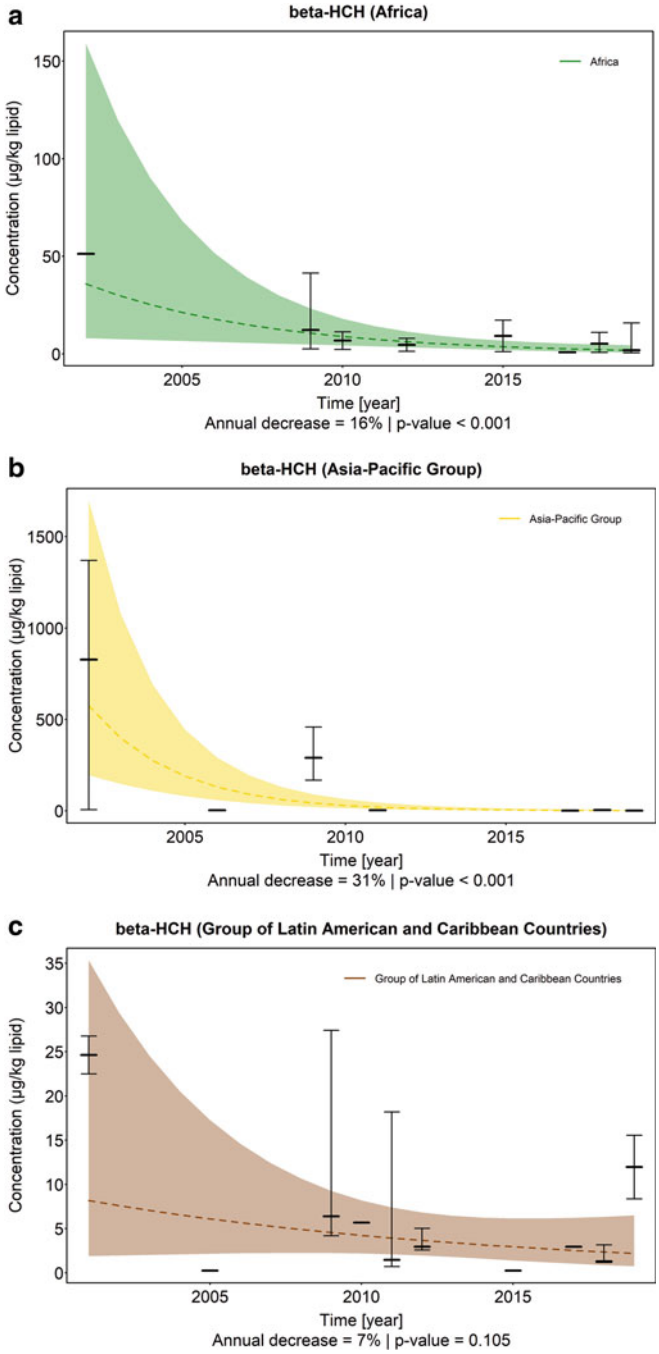

Fig. 13 (**a–f**) Theil–Sen exponential trends of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) worldwide and in the five UN regions. The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, whiskers show ranges between fifth and 95th percentiles

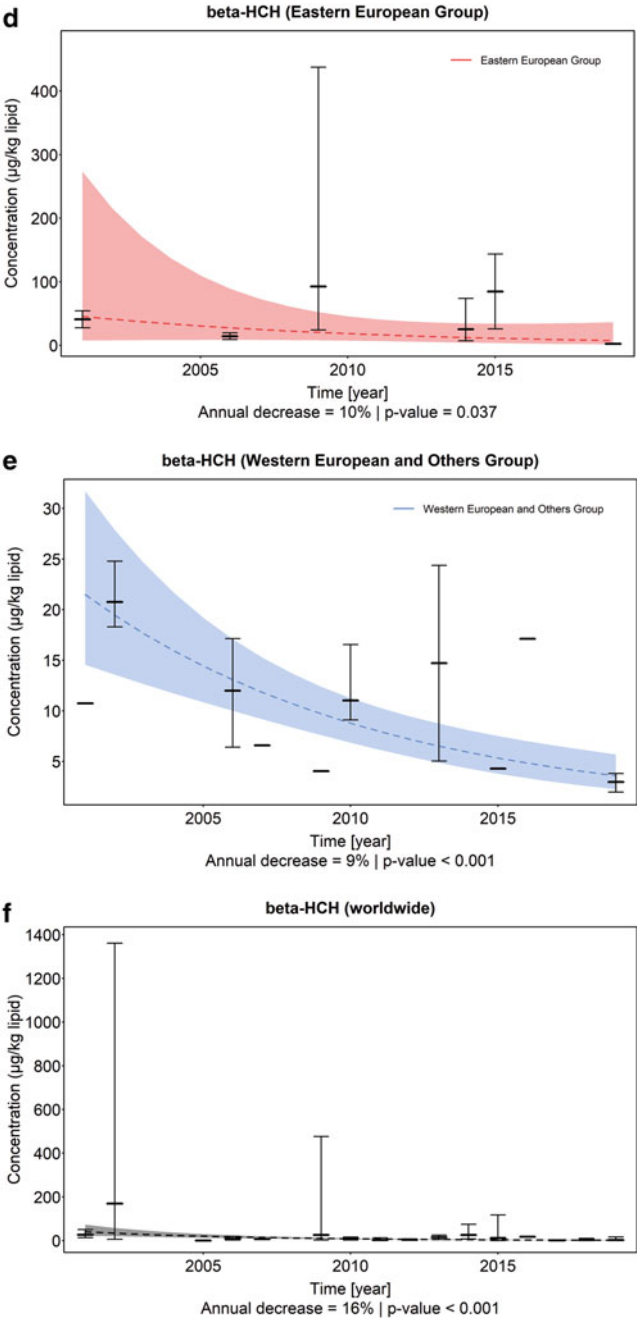

Fig. 13 (continued)

4.2 African Group

Figures 14 (for aggregated data) and 15 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of beta-HCH concentrations in 13 countries from Africa with repeated participation between 2000 and 2019. In Egypt, a reduction of 62% was observed from 51.3 μg/kg lipid in the 2000–2003 period to the 2016–2019 period. Most countries participated for the first time in the 2008–2011 period (range 1.9 μg/kg lipid to 48.3 μg/kg lipid; median 8.3 μg/kg lipid); in nearly all countries, beta-HCH concentrations fell on average by about 63% until the period 2016–2019 (decreases in the range between 43% and 92%). In Côte d'Ivoire, which was in the middle of the frequency distribution of beta-HCH concentrations in Africa, levels increased from 11.8 μg/kg lipid in 2010 to 2015 by 54%.

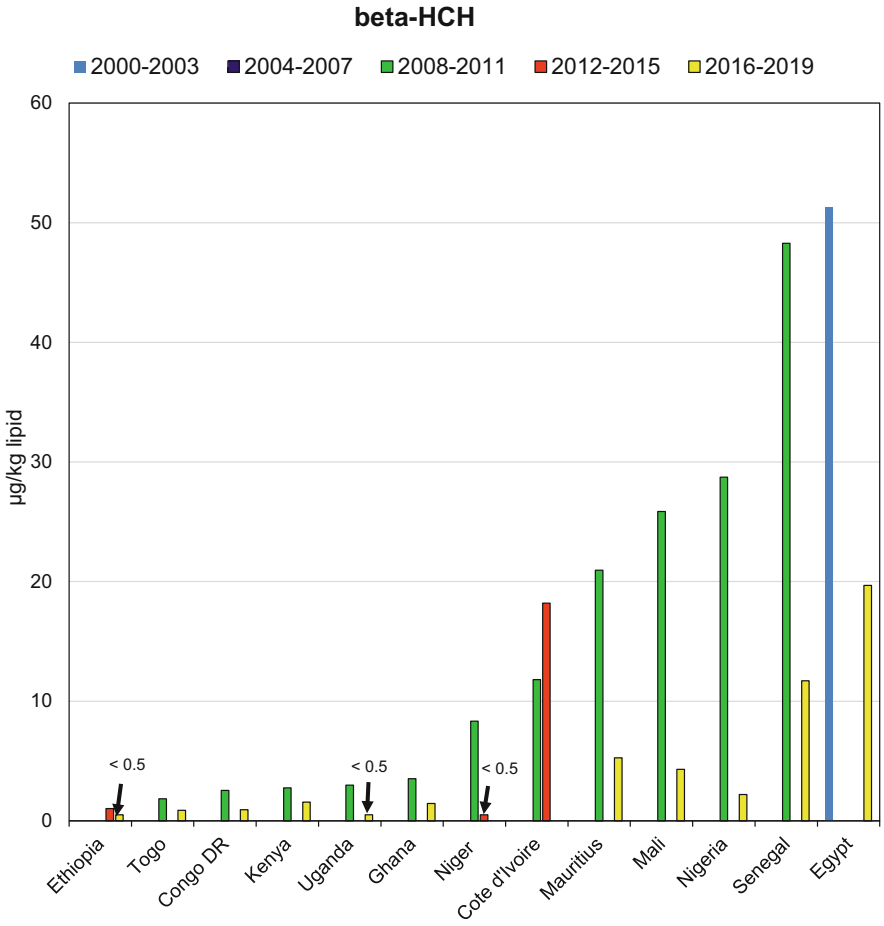

Fig. 14 Overview of the development of beta-HCH concentrations in human milk (expressed as μg beta-HCH/kg lipid; aggregated data) over time in African countries with repeated participation between 2000 and 2019

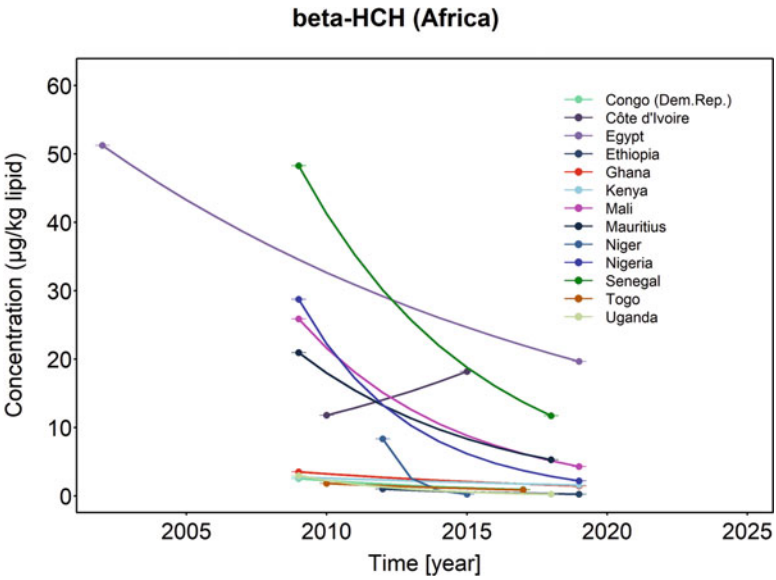

Fig. 15 Temporal tendencies of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in African countries with repeated participation between 2000 and 2019 using the Theil–Sen method

Table 9 Overall decrease (%) of beta-HCH concentrations in human milk per 1 year, 5 years and 10 years in African countries (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Trend *p*-value overall
Congo (DR)	11.8	46.6	71.5	1.000
Côte d'Ivoire	−9.0	−54.2	−137.8	1.000
Egypt	5.5	24.5	43.1	1.000
Ethiopia	18.2	63.4	86.6	1.000
Ghana	8.5	35.7	58.7	1.000
Kenya	5.5	24.5	43.1	1.000
Mali	16.4	59.2	83.4	1.000
Mauritius	14.2	53.6	78.4	1.000
Niger	68.9	99.7	100.0	1.000
Nigeria	22.6	72.3	92.3	1.000
Senegal	14.6	54.5	79.3	1.000
Togo	9.9	40.7	64.9	1.000
Uganda	24.1	74.8	93.6	1.000
Median	14.2	53.6	78.4	

The overall decreases per 1 year, 5 years and 10 years are given in Table 9. The limited number of samples did not allow to determine statistically significant decreases. In all African countries except Côte d'Ivoire, the decreases in the levels of beta-HCH

within a 10-year period were in the range between 43% and 100%. Similar decreases were found as well at the higher end of the frequency distribution of concentrations as at the lower end in the range of background contamination. The median of 78% is in line with the statistically significant ($p < 0.001$) decrease over 10 years for all African countries of 83% calculated by the Theil–Sen method (see Sect. 4.1).

4.3 Asia-Pacific Group

Figures 16 (for aggregated data) and 17a–b (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of beta-HCH concentrations in 8 countries from the Asia-Pacific Group with repeated participation between 2000 and 2019. Overall decrease rates per 1 year, 5 years and 10 years are given in Table 10.

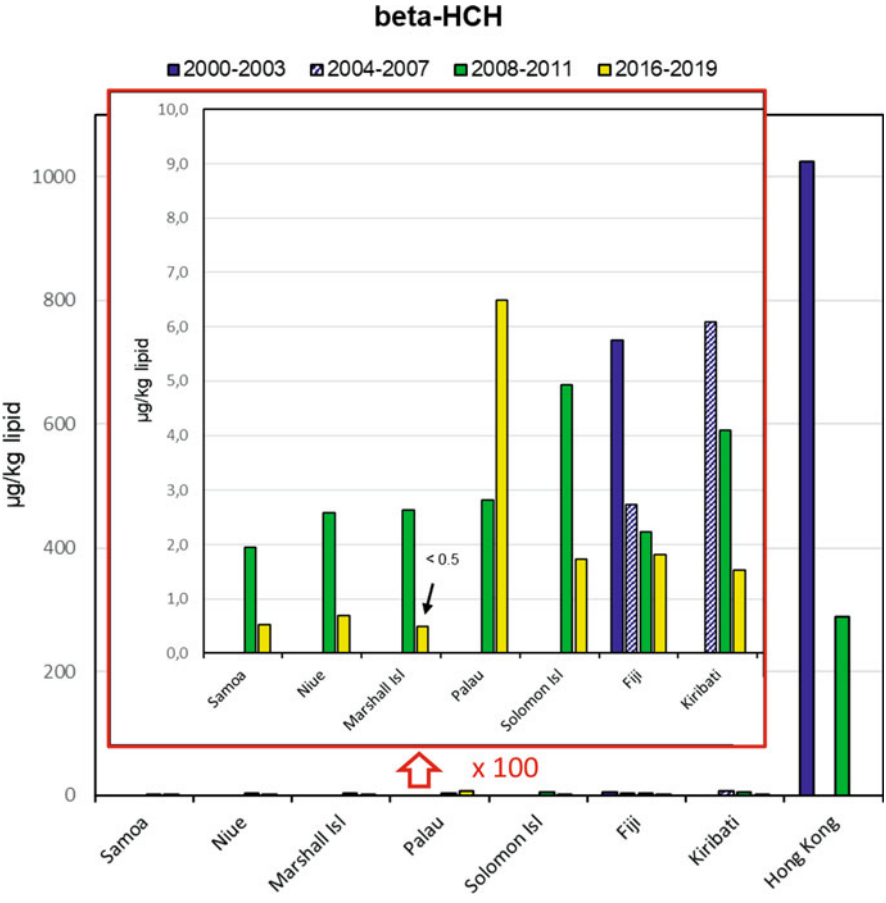

Fig. 16 Overview of the development of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid; aggregated data) over time in countries of the Asia-Pacific Group with repeated participation between 2000 and 2019

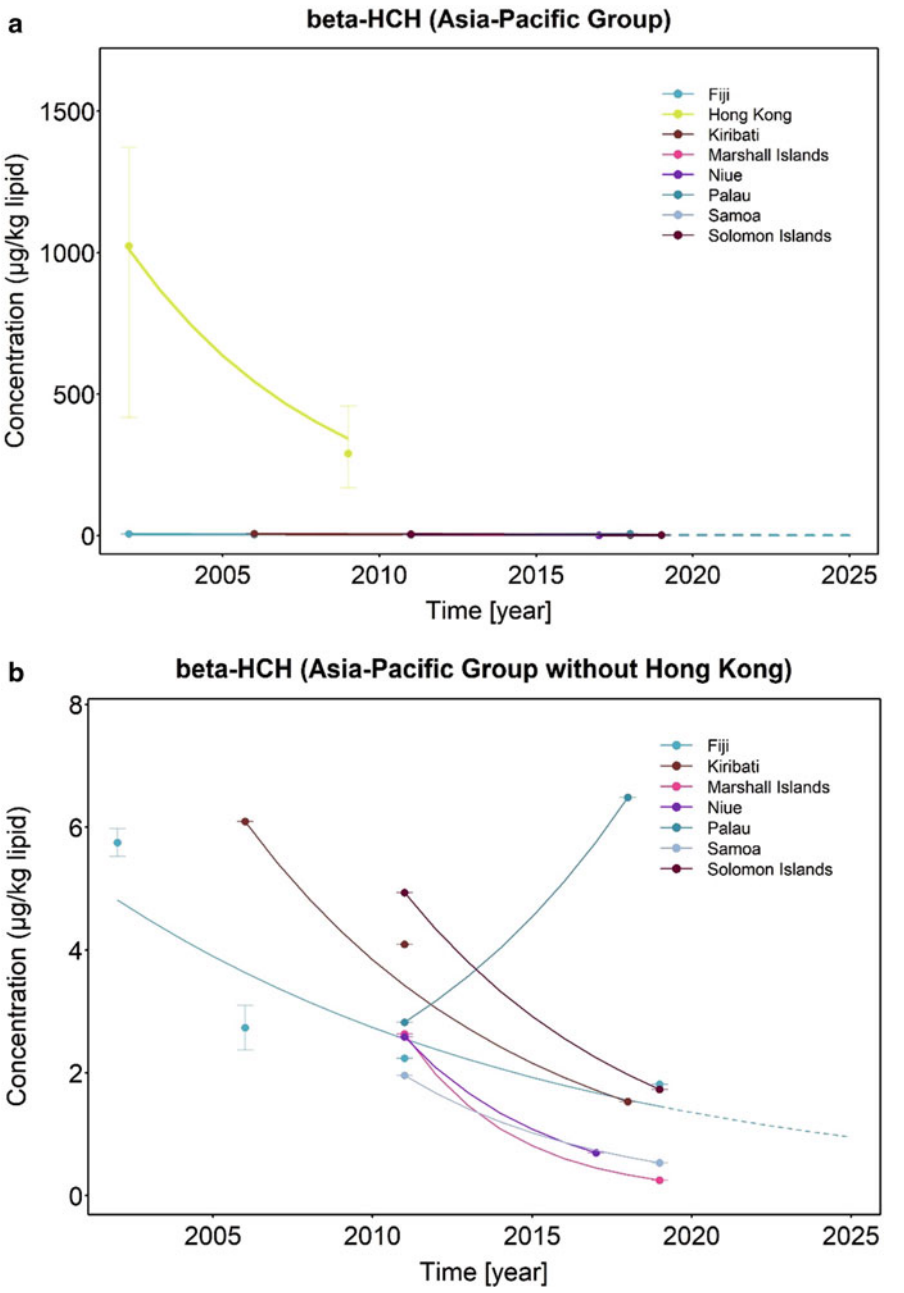

Fig. 17 (**a**–**b**) Temporal tendencies of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in (**a**) all countries of the Asia-Pacific Group and (**b**) Pacific Islands countries with repeated participation between 2000 and 2019 using the Theil–Sen method (with statistically significant time trends in Hong Kong SAR of China and Fiji)

Table 10 Overall decrease (%) of beta-HCH concentrations in human milk in countries of the Asia-Pacific Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025[μg/kg lipid]	Trend *p*-value overall
Fiji	6.8	29.7	50.6	0.95	< 0.001
Hong Kong	14.4	53.9	78.8		< 0.001
Kiribati	10.9	43.8	68.4		0.25
Marshall Isl	25.5	77.0	94.7		1.000
Niue	19.6	66.4	88.7		1.000
Palau	−12.6	−81.2	−228.4		1.000
Samoa	15.0	55.6	80.3		1.000
Solomon Isl	12.3	48.1	73.1		1.000
Median	13.3	51.1	76.1		

The highest concentration found in Hong Kong SAR of China in 2002 (1020 μg beta-HCH/kg lipid as the median of 10 pooled samples [Hedley et al. 2010]) decreased until 2009 by 78% to 290 μg/kg as median of 4 samples from different population groups (two age groups [above or below 30 years] which had been living in Hong Kong for either more or less than 10 years).

In comparison to these concentration ranges, beta-HCH levels in human milk from 7 Pacific Islands countries were lower by about a factor of 100 and more and in the range of low background contamination between <0.5 μg/kg lipid and 6 μg/kg lipid over the whole period between 2000 and 2019. With this, all countries of the Pacific region were among the countries with the lowest beta-HCH concentrations in human milk on a global level. As in the African countries, downtrends were also seen at these comparably low concentration ranges. In Fiji, a reduction of nearly 70% was observed from 5.8 μg beta-HCH/kg lipid in 2002 until 2019. Most other countries participated the first time in the period 2008–2011 and Kiribati in 2004–2007. In nearly all of these countries, a decrease in the range of 65–81% was observed in the following years, whereas in Palau, beta-HCH concentrations increased from the relatively low level of 2.8 μg/kg in 2002 to 6.5 μg/kg in 2019.

A statistically significant decrease in the levels of beta-HCH within a 10-year period of 79% was achieved for the initially high levels by Hong Kong SAR of China. For Fiji with a statistically significant decrease of 51% over 10 years and participation also in the 2016–2019 round, in addition the concentration in 2025 was estimated.

High reduction rates were also found in the range of lower background contamination of other Pacific Islands countries, with exception of Palau. However, due to the limited number of pooled samples per country, these decreases are not statistically significant (see subsection 2.3). Furthermore, the variation should be seen in context with the advised differentiation of levels above or—as in these cases—in the range of background contamination (approximately <5 μg beta-HCH/kg lipid), as explained in subsection 3.7.

4.4 Group of Latin American and Caribbean Countries (GRULAC)

Figures 18 (for aggregated data) and 19 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of beta-HCH concentrations in 9 Latin American and Caribbean countries with repeated participation between 2000 and 2019. In Brazil, a decrease of median concentrations by 88% was found from 2001 to 2012. In Haiti, beta-HCH concentrations in human milk remained constantly low in the range of the limit of quantification (0.5 μg/kg) from 2004 to 2015. The other countries participated for the first time in the period 2008–2011. In nearly all of them, a decrease was observed in the following years (range 41–67%). In Jamaica and Mexico with beta-HCH concentrations in the lower background range below 3 μg/kg lipid, the levels remained quite constant.

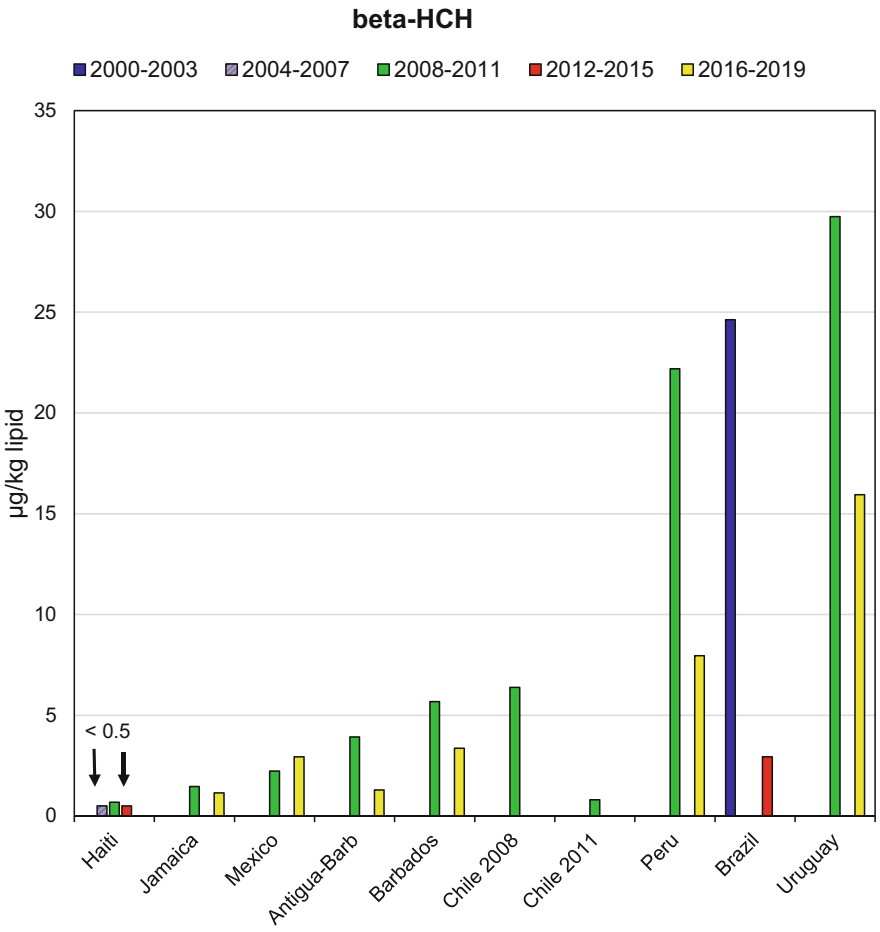

Fig. 18 Overview of the development of beta-HCH concentrations in human milk (expressed as μg beta-HCH/kg lipid; aggregated data) over time in Latin American and Caribbean Countries with repeated participation between 2000 and 2019

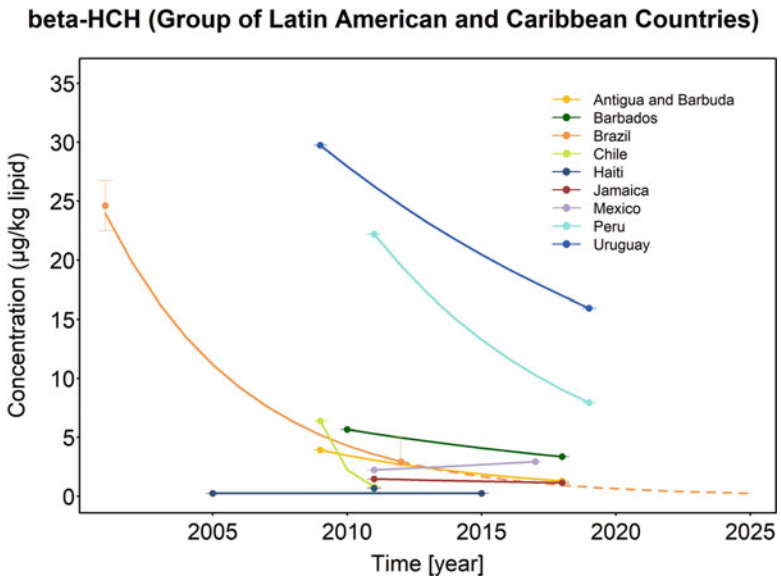

Fig. 19 Temporal tendencies of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in Latin American and Caribbean Countries with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Brazil)

These findings are also reflected in the overall decreases per 1 year, 5 years and 10 years (Table 11). In all countries except Brazil, the limited number of pooled samples did not allow to determine statistically significant reduction rates. A decrease in the beta-HCH levels within a 10-year period between 46% and 85% was seen in countries at the upper end of the frequency distribution (Brazil, Peru and

Table 11 Overall decrease (%) of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) per 1 year, 5 years and 10 years in Latin American and Caribbean Countries and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [µg/kg lipid]	Trend *p*-value overall
Antigua-Barb.	11.6	46.1	71.0		1.000
Barbados	6.3	27.9	48.0		1.000
Brazil	17.4	61.5	85.2	0.25	0.031
Chile	64.6	99.4	100.0		1.000
Haiti	0.0	0.0	0.0		1.000
Jamaica	3.3	15.7	28.9		1.000
Mexico	−4.7	−25.9	−58.6		1.000
Peru	12.0	47.3	72.2		1.000
Uruguay	6.0	26.8	46.4		1.000
Median	6.3	27.9	48.0		

Uruguay), whereas in the other countries with beta-HCH concentrations mostly in the range of the background contamination (<5 µg/kg), a wide range of reduction rates was found. Therefore, as explained above for the Asia-Pacific countries, a differentiation of levels above or in the range of background contamination seems to be advised. The calculated tendencies for Haiti, Mexico and Jamaica show the variation at low background levels over time.

4.5 Eastern European Group

Figures 20 (for aggregated data) and 21 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of beta-HCH concentrations in 6 countries of the Eastern European Group with

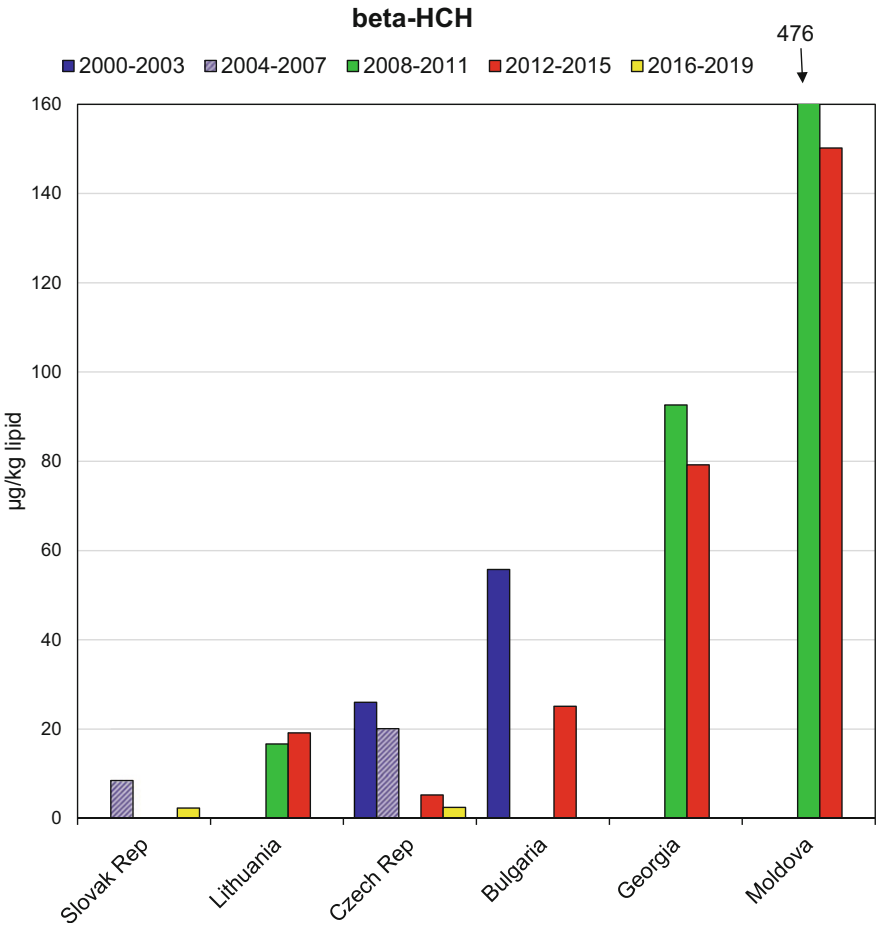

Fig. 20 Overview of the development of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid; aggregated data) over time in countries of the Eastern European Group with repeated participation between 2000 and 2019

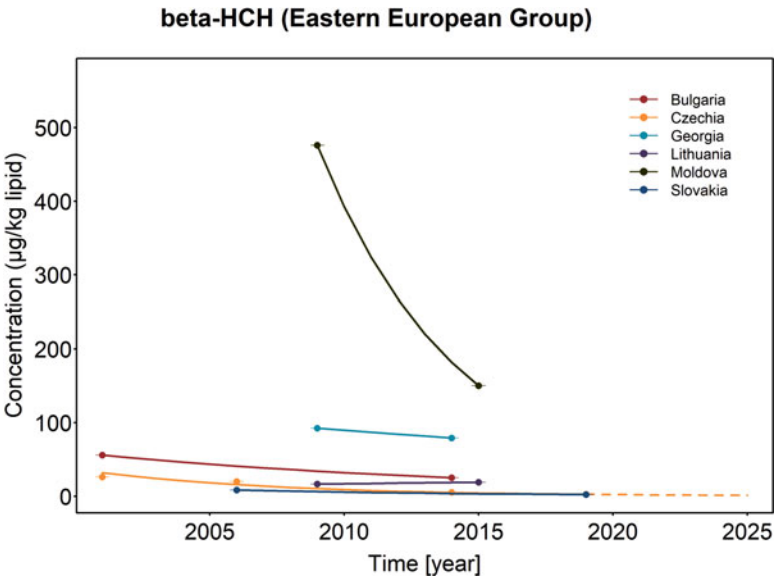

Fig. 21 Temporal tendencies of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in countries of the Eastern European Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Czechia)

Table 12 Overall decrease (%) of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in countries of the Eastern European Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Bulgaria	5.9	26.4	45.8		1.000
Czechia	13.2	50.6	75.6	1.08	0.031
Georgia	3.1	14.5	26.8		1.000
Lithuania	−2.4	−12.4	−26.4		1.000
Moldova	17.5	61.8	85.4		1.000
Slovakia	9.6	39.5	63.4		1.000
Median	7.8	33.3	55.5		

repeated participation between 2000 and 2019. A continuous decrease is observed in nearly all countries over all periods. Most data points are available for the Czech Republic with four participations between the 2001 and 2019. Here, beta-HCH concentrations in human milk fell from 26 µg/kg lipid by 91% until 2019. The highest concentration found in Moldova in 2009 (476 µg beta-HCH/kg lipid) decreased by 68% until 2015.

The overall decreases per 1 year, 5 years and 10 years are given in Table 12. A statistically significant decrease over 10 years of 76% was observed in Czechia; also

for this country, a prognosis of the estimated beta-HCH concentration in 2025 was calculated. In all other countries, the limited number of samples did not allow to determine statistically significant reduction rates. For most Eastern European countries, the decrease over 10 years was between 27% and 85%. In Lithuania, beta-HCH concentrations slightly increased from 16.6 µg/kg lipid in 2009 by 15% until 2014.

4.6 Western European and Others Group (WEOG)

Figures 22 (for aggregated data) and 23 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes for beta-HCH in 8 countries of the Western European and Others Group with repeated participation between 2000 and 2019. In Germany, which covers the entire period between 2000 and 2019, a decrease of beta-HCH concentrations in human milk from 19.4 µg/kg lipid in 2002 by 84% to 2019 was achieved, with a statistically significant decrease over 10 years of 67%. Based on this, a prognosis of the estimated concentration in 2025 was derived for Germany (Table 13).

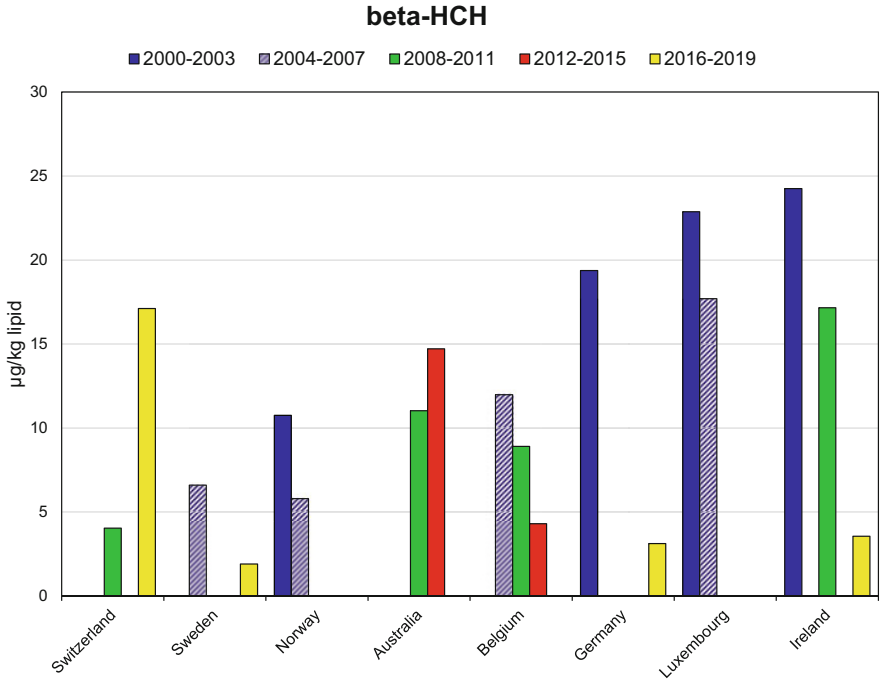

Fig. 22 Overview of the development of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid; aggregated data) over time in countries of the Western European and Others Group with repeated participation between 2000 and 2019

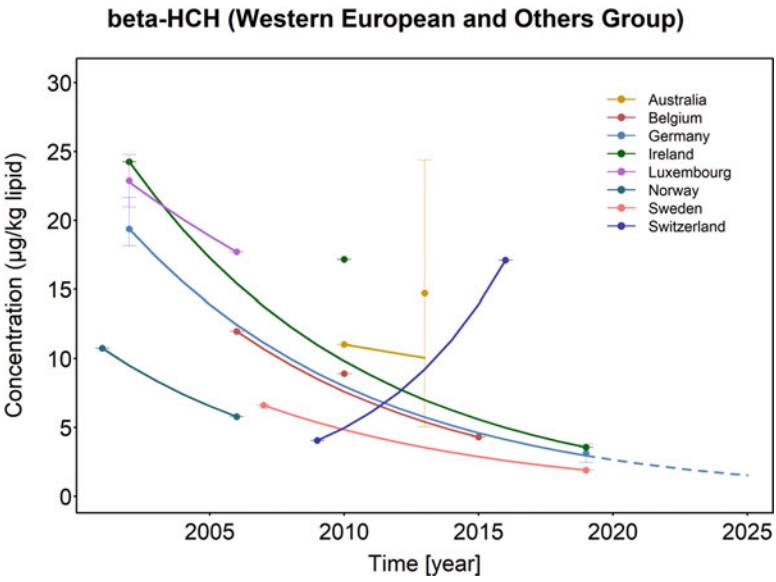

Fig. 23 Temporal tendencies of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in countries of the Western European and Others Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Germany)

Table 13 Overall decrease (%) of beta-HCH concentrations in human milk (expressed as µg beta-HCH/kg lipid) in countries of the Western European and Others Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Australia	3.0	14.2	26.5		1.000
Belgium	10.8	43.4	68.0		0.250
Germany	10.5	42.5	66.9	1.52	0.008
Ireland	10.7	43.2	67.7		0.250
Luxembourg	6.1	27.0	46.7		0.500
Norway	11.6	46.1	71.0		1.000
Sweden	9.9	40.5	64.6		1.000
Switzerland	−22.9	−180.5	−686.6		1.000
Median	10.2	41.5	65.8		

In all other countries, the limited number of pooled samples did not allow to determine statistically significant decreases. The median of the reduction rates per 10 years for five Western European countries (Belgium, Ireland, Luxembourg, Norway and Sweden) was 68% (range 47–71%). In Switzerland, beta-HCH concentrations increased from 4.0 µg/kg in 2009 to 17.1 µg/kg in 2016.

For Australia, data are available for 2010 (one pooled sample with 11 μg beta-HCH/kg lipid) and 2013 (two pooled samples with 25.5 μg beta-HCH/kg lipid and 4.0 μg beta-HCH/kg lipid, respectively). Therefore, the median of the two pooled samples from 2013 shows an increase, whereas based on the Theil–Sen method an overall decrease is calculated with regard to the two very different levels found in 2013 (Table 13; Fig. 23).

4.7 Dependence of Decrease (Decrease Rate Constants) on Concentration

Beta-HCH concentrations in human milk in the 44 countries with repeated participation between 2000 and 2019 comprise a wide range between <0.5 μg beta-HCH/kg lipid and 1020 μg beta-HCH/kg lipid. This was grouped into five ranges (<5; 5–10; 10–20; 20–100; >100 μg beta-HCH/kg lipid) to check the dependence of the decrease (*decrease rate constants*) on the "initial" (the first measured) concentration in a country. The lower end of the frequency distribution below 5 μg beta-HCH/kg lipid is considered as (low) background contamination. The upper end with the two highest levels (476 μg/kg; 2010 μg/kg) is related to a more recent use.

Table 14 shows, that the reduction rates over 10 year for beta-HCH concentrations above 20 μg/kg lipid vary less than in the lower part: For all samples above 20 μg/kg, only downward trends were observed, with a decrease of about 80% over 10 years for the two samples with highest concentrations (>100 μg/kg) and a wide variation in the range 20–100 μg/kg. In particular, samples in the low background range below 5 μg beta-HCH/kg lipid show a high variation of the reduction rates (range between −690% and 95%, median 68%, for 15 of the 16 pooled samples in this range below 5 μg beta-HCH/kg lipid, but above LOQ [0.5 μg/kg lipid] and one pooled sample below LOQ). At lower background levels, many factors, e.g. contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally. It might be concluded that the large variation of reduction rates for beta-HCH concentrations in this lower background range limits the applicability of this parameter for assessments at the country-level, but allows a more general assessment of the temporal trends of background contamination.

Figure 24 illustrates this dependence of decrease rates over 10 years in 44 countries on the concentration range with repeated participation between 2000 and 2019.

Table 14 Overall decrease (%) of beta-HCH concentrations in human milk (μg/kg lipid) over 10 years calculated by the Theil–Sen method and their dependence on the concentration range. Negative decreases are to be read as increase

	<5 μg/kg	5–10 μg/kg	10–20 μg/kg	20–100 μg/kg	>100 μg/kg
N	16	7	6	13	2
min	−687	48	−138	27	79
median	68	65	47	72	82
max	95	100	71	92	85

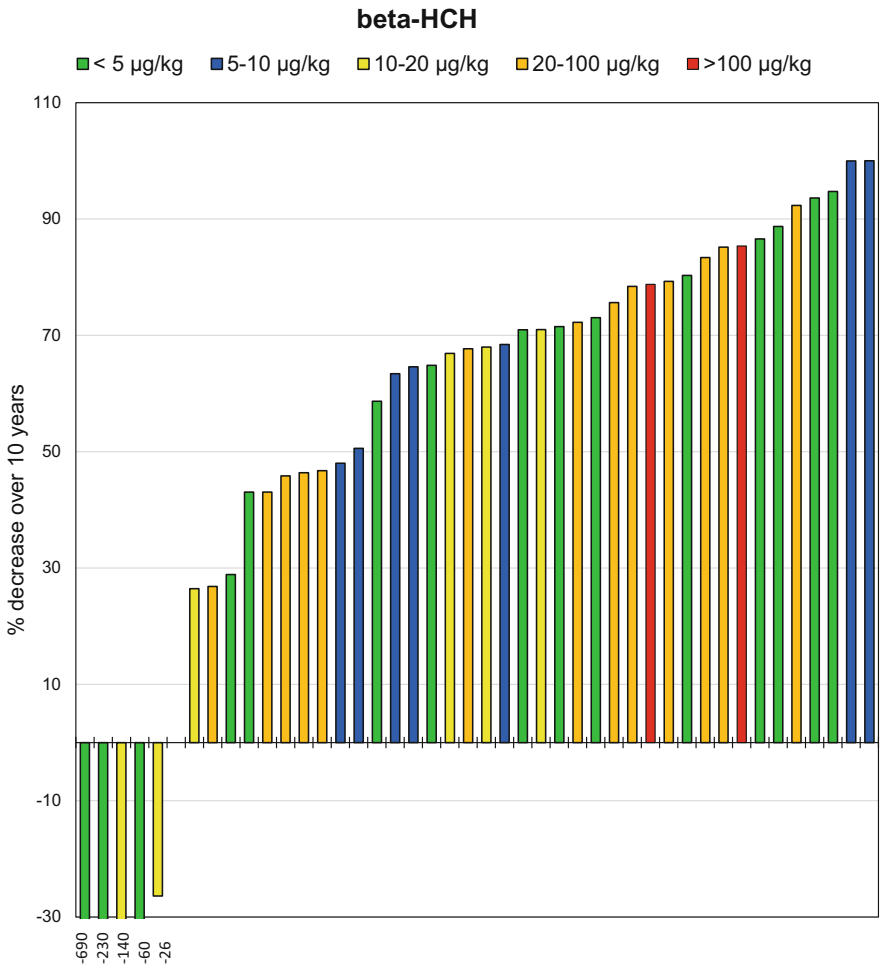

Fig. 24 Dependence of the decrease over 10 years (calculated by the Theil–Sen method) for beta-HCH on concentrations in human milk in 44 countries with repeated participation between 2000 and 2019, with differentiation into five ranges of concentration (<5; 5–10; 10–20; 20–100; >100 µg/kg lipid)

5 Hexachlorobenzene (HCB)

5.1 Global Level and Comparison Between UN Regional Groups

The maximum levels and therefore the ranges of HCB were much lower than found for DDT and beta-HCH, with a minimum of about 1–2 µg/kg lipid found in some countries and a maximum of 154 µg/kg lipid found in 2009 in an Eastern European

Table 15 Overall decrease (%) of the HCB concentration in human milk (expressed as µg/kg lipid) in the five UN Regional Groups and worldwide (computed using all samples from countries with repeated participation)

UN Regional Groups	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall[a]
		Theil–Sen method	Median method	Theil–Sen method	Median method	
Africa	13	1.2	1.1	11.3	10.2	0.002
Asia-Pacific	8	10.5	6.5	67.2	49.1	<0.001
Latin America and Caribbean	9	3.6	6.6	30.7	49.6	<0.001
Eastern Europe	6	6.1	6.9	46.6	51.2	0.037
Western Europe and Others	8	5.8	1.9	44.8	17.3	<0.001
Global	44	8.0	5.8	56.7	44.8	<0.001

[a]For Theil–Sen method

country. The median of 119 country results from 44 countries with repeated participation was 6.1 µg/kg lipid. This is the same range as found in 134 country results (based on aggregated data) of 82 countries (as total number regardless the number of participations between 2000 and 2019), with a similar median of 5.1 µg/kg. Many results were in the lower background range, such as the results of all 19 countries from Africa over the whole period (Malisch et al. 2023b). As explained above (subsections 3.7 and 4.7), some fluctuation of decrease rates within a 10-year period might be expected when concentrations tend to level out.

In the African Group with all pooled samples collected over time being in the background range of 5 µg HCB/kg lipid or below, the overall decrease within 10 years was 11% calculated by the Theil–Sen method and 10% by the median method. Overall decreases between 30% and 67% were calculated by the Theil–Sen method for the other UN Regional Groups, and 57% on a global level. Overall decreases calculated by the median method are in the range between 17% and 50% in these UN Regional Groups, with 48% as median of the five UN regions and 26% as median of 44 countries (Table 15). The variation between countries in the UN Regional Groups is shown in the following subsections.

The exponential trends of HCB concentrations derived by the Theil–Sen method in the five UN regional groups and worldwide are illustrated in Fig. 25a–f. Again, these figures are normalized according to the maximum concentration found in the respective UN Regional Groups. Thus, the different scales illustrate the different ranges between and within the UN Regional Groups. For a detailed discussion of the regional data, see the following subsections 5.2–5.6.

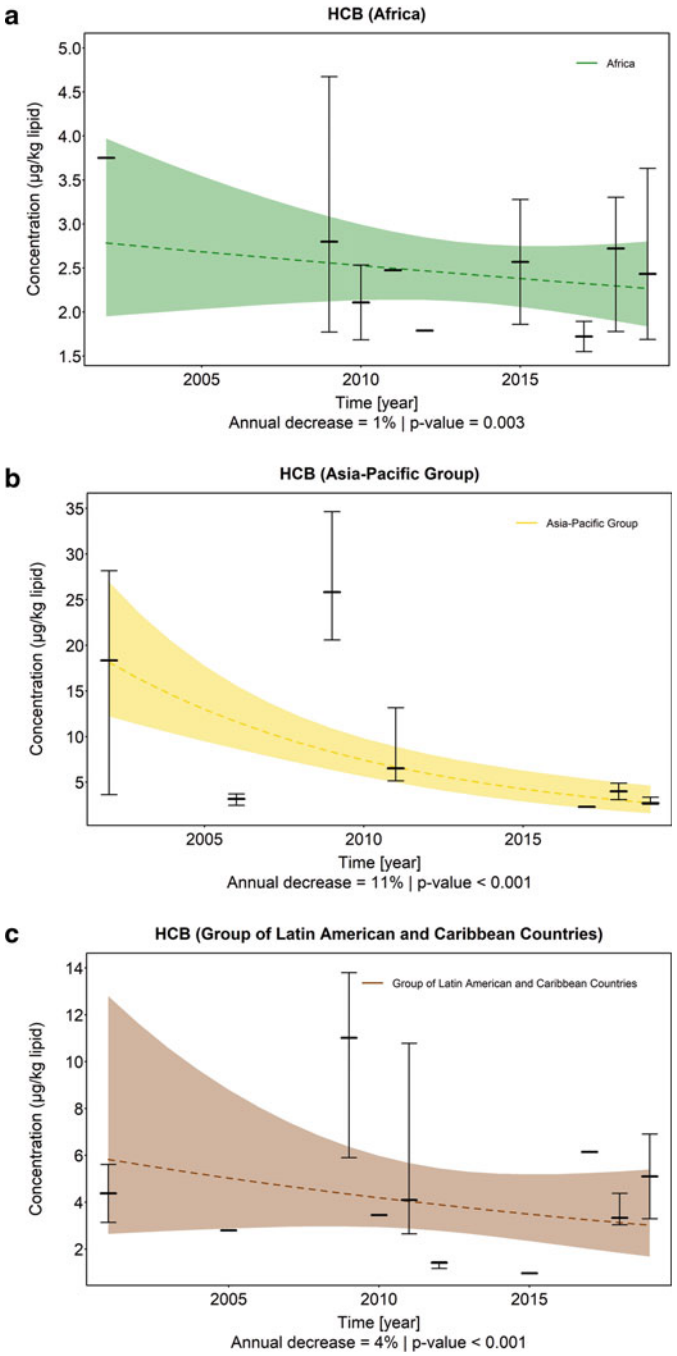

Fig. 25 (**a–f**) Theil–Sen exponential trends of the HCB concentration in human milk (expressed as µg/kg lipid) worldwide and in the five UN regions. The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, whiskers show ranges between fifth and 95th percentiles

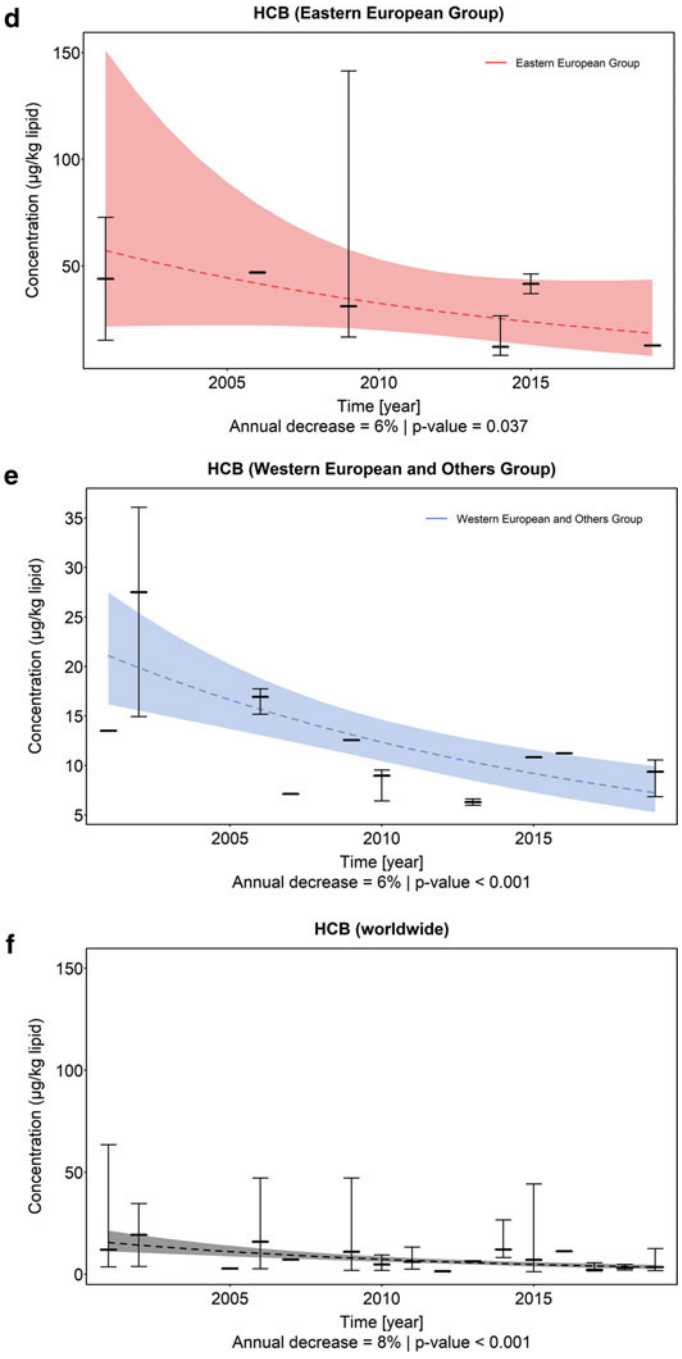

Fig. 25 (continued)

5.2 African Group

All 13 countries from Africa with repeated participation were at all times in the low background range below 5 μg HCB/kg lipid. In most countries, even these comparably low levels seemed to decrease over time, whereas in few countries, an increase seemed to be observed. However, it is more likely that these findings reflect the HCB variation in the lower background range over time, which might be levelling out and quite stable at low concentrations of approximately 2–3 μg HCB/kg lipid.

Figures 26 (for aggregated data) and 27 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of HCB. Table 16 compiles the overall decreases calculated by the Theil–

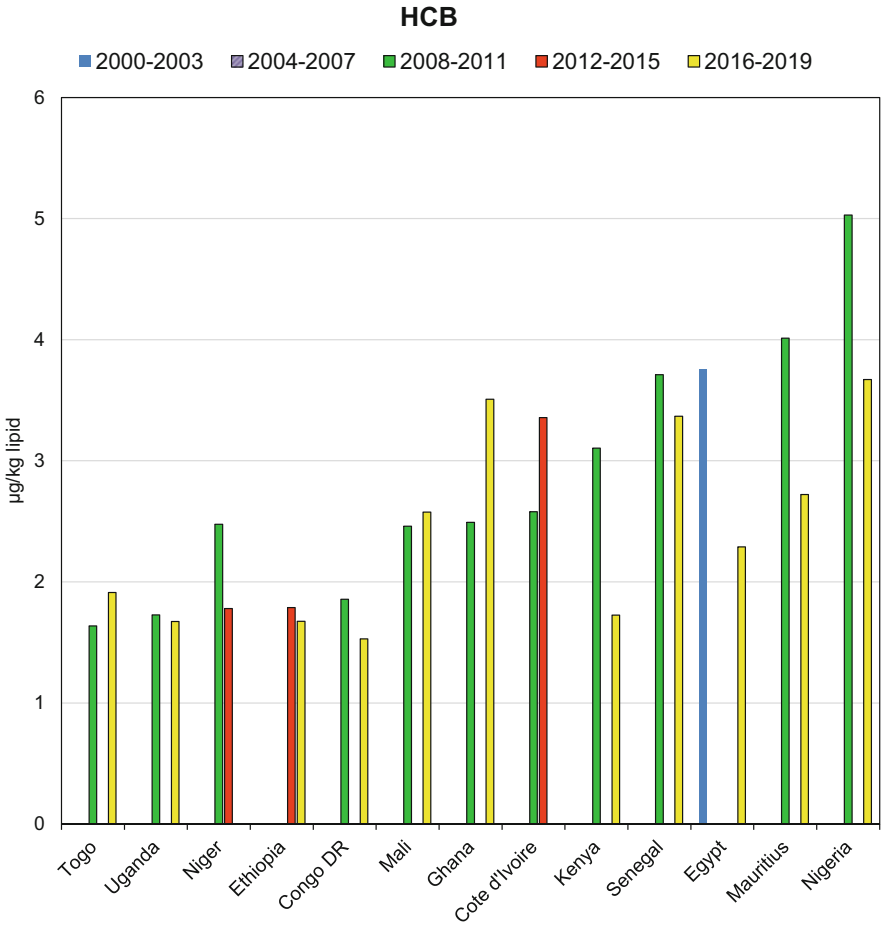

Fig. 26 Overview of the development of the HCB concentration in human milk (expressed as μg/kg lipid; aggregated data) over time for African countries with repeated participation between 2000 and 2019

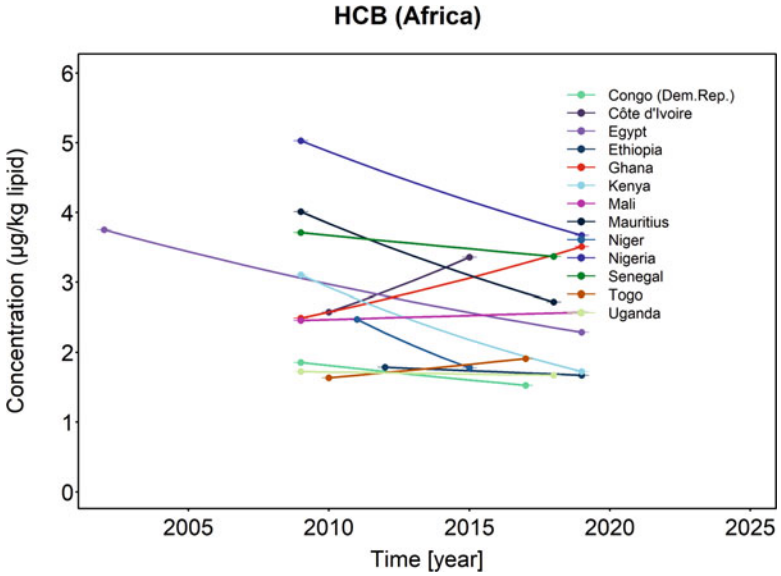

Fig. 27 Temporal tendencies of the HCB concentration in human milk (expressed as µg/kg lipid) in African countries with repeated participation between 2000 and 2019 using the Theil–Sen method

Table 16 Overall decrease (%) of the HCB concentration in human milk in African countries per 1 year, 5 years and 10 years (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Trend *p*-value overall
Congo (DR)	2.4	11.4	21.5	1.000
Côte d'Ivoire	−5.4	−30.1	−69.3	1.000
Egypt	2.9	13.5	25.2	1.000
Ethiopia	0.9	4.6	9.0	1.000
Ghana	−3.5	−18.7	−40.8	1.000
Kenya	5.7	25.4	44.4	1.000
Mali	−0.5	−2.3	−4.7	1.000
Mauritius	4.2	19.4	35.0	1.000
Niger	7.9	33.8	56.1	1.000
Nigeria	3.1	14.6	27.0	1.000
Senegal	1.1	5.2	10.2	1.000
Togo	−2.2	−11.7	−24.9	1.000
Uganda	0.4	1.7	3.5	1.000
Median	1.1	5.2	10.2	

Sen method. The limited number of samples did not allow to determine statistically significant decrease rates.

5.3 Asia-Pacific Group

Figures 28 (for aggregated data) and 29 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of HCB concentrations in 8 countries from the Asia-Pacific Group with repeated participation between 2000 and 2019. Table 17 compiles the overall decreases

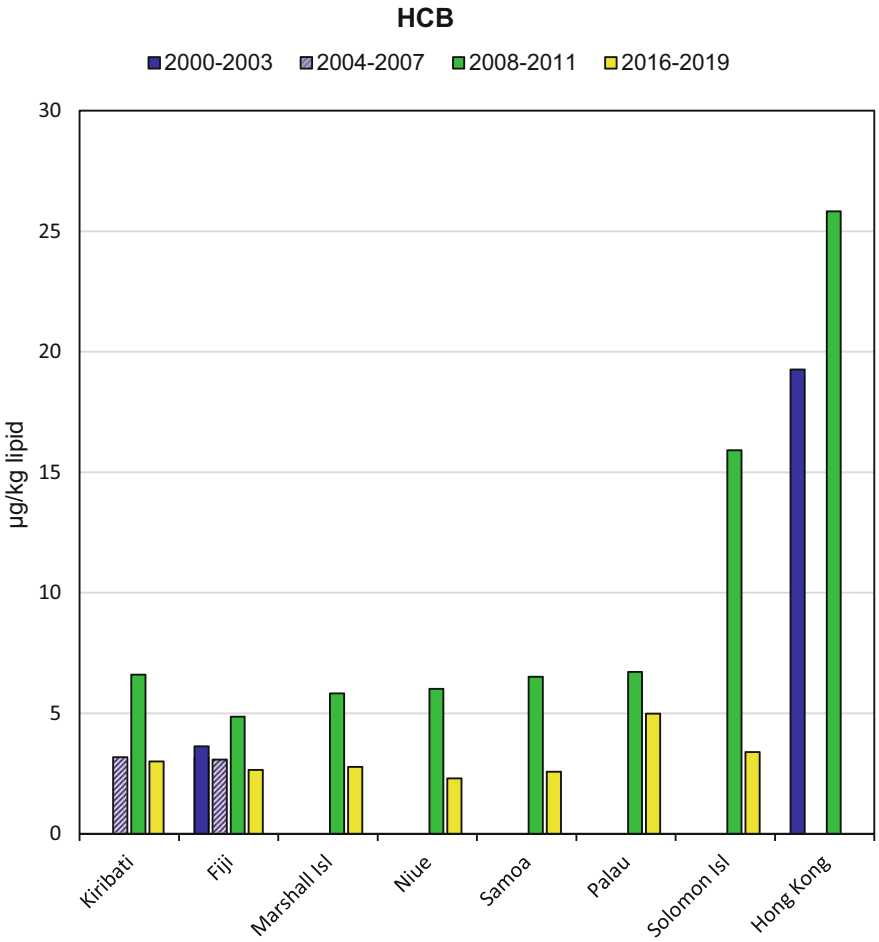

Fig. 28 Overview of the development of the HCB concentration in human milk (expressed as µg/kg lipid; aggregated data) over time in countries of the Asia-Pacific Group with repeated participation between 2000 and 2019

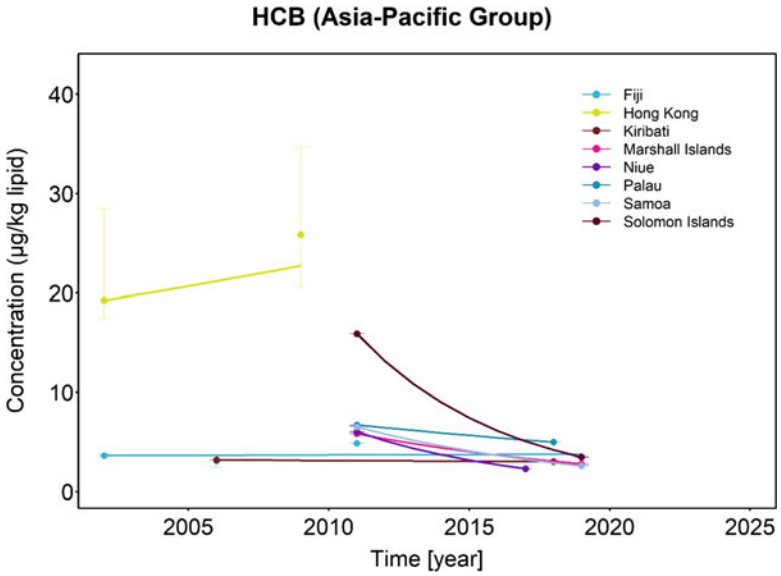

Fig. 29 Temporal tendencies of the HCB concentration in human milk (expressed as µg/kg lipid) over time in countries of the Asia-Pacific Group with repeated participation between 2000 and 2019 using the Theil–Sen method

Table 17 Overall decrease (%) of the HCB concentration in human milk in countries of the Asia-Pacific Group per 1 year, 5 years and 10 years (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Trend *p*-value overall
Fiji	−0.2	−1.1	−2.2	0.950
Hong Kong	−2.4	−12.6	−26.8	< 0.001
Kiribati	0.5	2.3	4.6	1.000
Marshall Islands	8.8	37.0	60.3	1.000
Niue	14.8	55.1	79.9	1.000
Palau	4.2	19.2	34.8	1.000
Samoa	11.0	44.0	68.7	1.000
Solomon Islands	17.4	61.5	85.2	1.000
Median	6.5	28.1	47.6	

calculated by the Theil–Sen method. In nearly all countries, the limited number of samples did not allow to determine statistically significant decreases.

From the Asian part of this UN regional group, Hong Kong SAR of China participated twice, with an increase of the median concentrations from 2002 (19.3 μg HCB/kg lipid as median of 10 pooled samples [Hedley et al. 2010]) to the 2009 level of 25.8 μg/kg (median of 4 samples from different population subgroups). These summarizing temporal trends could be further differentiated: Two subgroups of 2009 with residents who had been living in Hong Kong for 10 years or more had comparable HCB concentrations (20.5–21.0 μg/kg) to 2002, whereas two subgroups of 2009 who had been living in Hong Kong for less than 10 years had HCB concentrations of 30–35 μg/kg.

Fiji participated four times between 2002 and 2019 and Kiribati three times between 2006 and 2018. Over the entire period, the HCB concentrations remained quite stable in the range of background contamination, i.e. around 3 μg/kg in Fiji, 2002 and 2019, and Kiribati, 2006 and 2018. The highest HCB concentration found in Solomon Islands in 2011 (15.9 μg/kg) was reduced by 79% until 2018. The other four countries of the Pacific Islands had HCB concentrations in human milk in the 2008–2011 period around 6–7 μg/kg and had overall decrease rates per 10 years between 35% and 80%.

5.4 Group of Latin American and Caribbean Countries (GRULAC)

Figures 30 (for aggregated data) and 31 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of HCB concentrations in 9 Latin American and Caribbean countries with repeated participation between 2000 and 2019. Table 18 compiles the overall decreases calculated by the Theil–Sen method. In all countries except Brazil, the limited number of samples did not allow to determine statistically significant decrease rates.

In all countries, HCB concentrations in human milk decreased over time. In Haiti, the level of 2.8 μg HCB/kg lipid in 2004 was reduced by 65% until 2015. In Brazil, a decrease of the median concentration of 4.4 μg/kg in 2001 by 68% was found until 2012. Most other countries participated for the first time in the period 2008–2011. In all of these countries, a decrease was observed until the 2016–2019 period (range 5–50%).

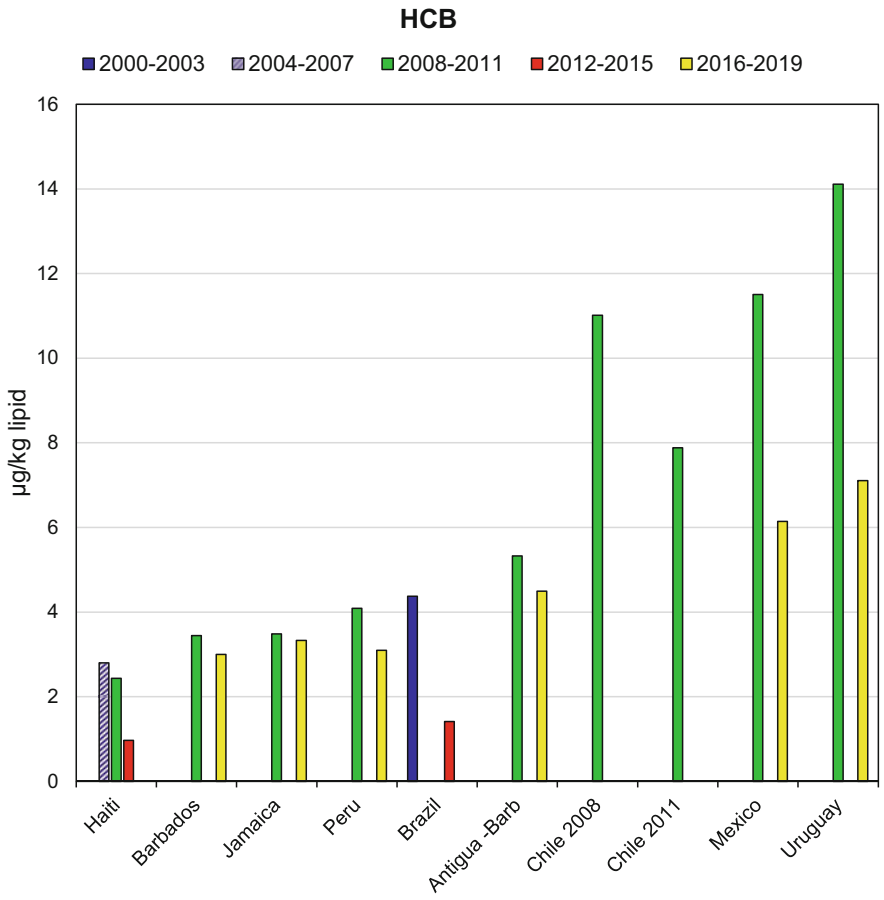

Fig. 30 Overview of the development of the HCB concentration in human milk (expressed as µg/kg lipid; aggregated data) over time in countries of the Group of Latin American and Caribbean Countries with repeated participation between 2000 and 2019

For countries with first participation in the 2008–2011 period and HCB concentrations in human milk in the range of the background contamination around or below 5 µg/kg (Antigua-Barbuda, Barbados, Jamaica and Peru), the overall decrease per 10 years was lower (range 6.1% to 29.5%) than for the countries with higher HCB levels in this period (range of HCB concentrations in Chile, Mexico and Uruguay: 8–14 µg/kg; decrease: range 50–81%).

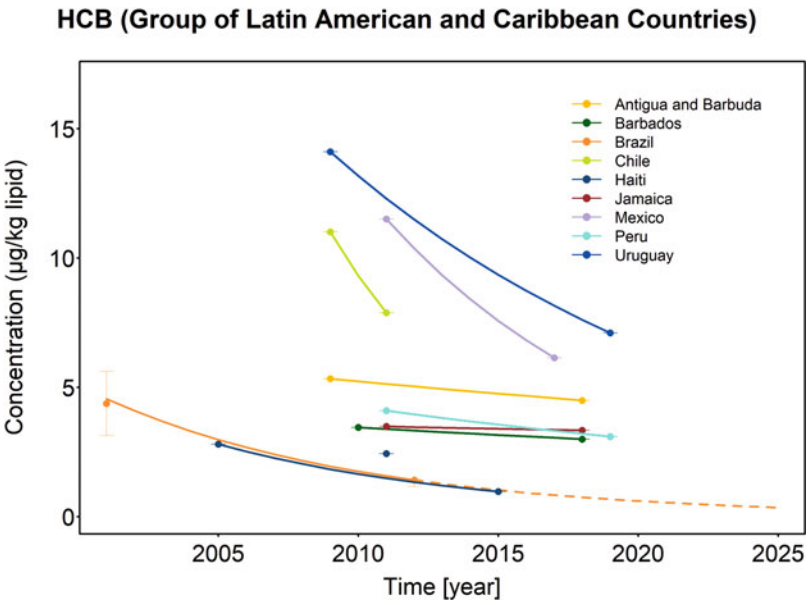

Fig. 31 Temporal tendencies of the HCB concentration in human milk (expressed as µg/kg lipid) in countries of the Group of Latin American and Caribbean Countries with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Brazil)

Table 18 Overall decreases (%) of the HCB concentration in human milk in countries of the Group of Latin American and Caribbean Countries per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Antigua-Barb.	1.9	9.0	17.2		1.000
Barbados	1.7	8.4	16.0		1.000
Brazil	10.1	41.3	65.5	0.35	0.031
Chile	15.4	56.7	81.3		1.000
Haiti	10.1	41.1	65.4		0.250
Jamaica	0.6	3.1	6.1		1.000
Mexico	9.9	40.7	64.9		1.000
Peru	3.4	16.0	29.5		1.000
Uruguay	6.6	29.0	49.6		1.000
Median	6.6	29.0	49.6		

5.5 Eastern European Group

Figures 32 (for aggregated data) and 33 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of HCB concentrations in 6 countries of the Eastern European Group with repeated participation between 2000 and 2019. Table 19 compiles the overall decreases calculated by the Theil–Sen method. In all countries except Czechia, the limited number of samples did not allow to determine statistically significant decreases.

A wide range of concentrations was observed with a maximum of 154 µg/kg found in Moldova, 2009. These concentrations decreased considerably by 70% until 2015. Czechia participated four times between 2000 and 2019; here a continuous downtrend was found from 76 µg HCB/kg lipid in 2001 with a reduction by 84% until 2019.

The overall reduction rates were lower in countries with initially lower HCB levels (Bulgaria, 2001: 12 µg/kg; Georgia, 2009: 15 µg/kg; decrease over 10 years:

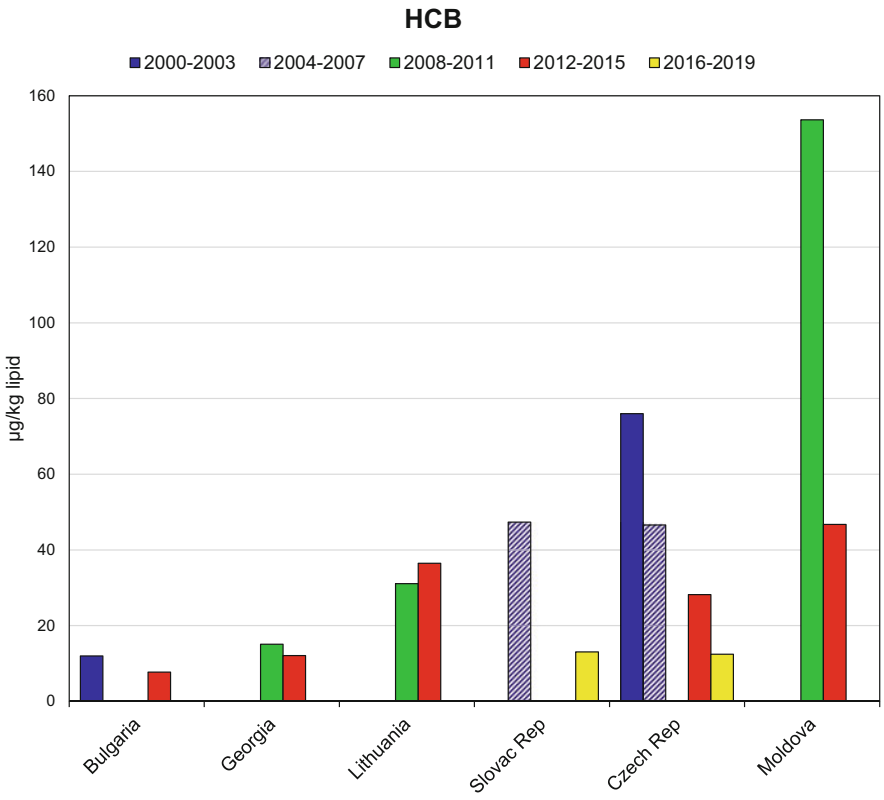

Fig. 32 Overview of the development of the HCB concentration in human milk (expressed as µg/kg lipid; aggregated data) over time in countries of the Eastern European Group with repeated participation between 2000 and 2019

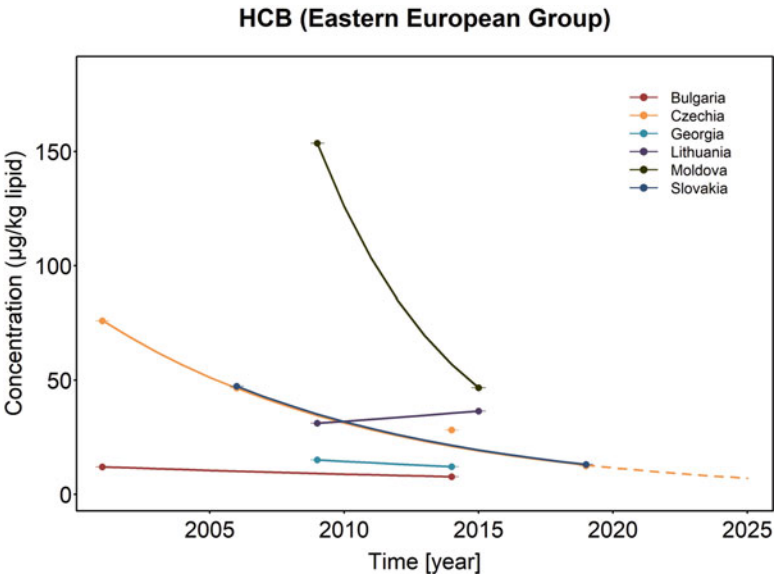

Fig. 33 Temporal tendencies of the HCB concentration in human milk in countries of the Eastern European Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Czechia)

Table 19 Overall decrease (%) of the HCB concentration in human milk (expressed as µg/kg lipid) in countries of the Eastern European Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Bulgaria	3.3	15.7	28.9		1.000
Czechia	9.4	39.1	62.9	7.06	0.031
Georgia	4.3	19.9	35.8		1.000
Lithuania	−2.7	−14.2	−30.5		1.000
Moldova	18.0	62.9	86.2		1.000
Slovakia	9.4	39.1	62.9		1.000
Median	6.9	30.1	51.2		

29% and 36%, respectively) than in countries with higher HCB levels (Czechia, Moldova, Slovakia; range of HCB concentrations: 47–154 µg/kg; decrease over 10 years: 63–86%). Only in Lithuania were the HCB concentrations increased slightly from 31.1 µg/kg in 2009 by 17% until 2015.

5.6 Western European and Others Group (WEOG)

Figures 34 (for aggregated data) and 35 (comprising all individual pooled samples and assuming exponential trends, see subsection 2.3) illustrate the temporal changes of HCB concentrations in 8 countries of the Western European and Others Group with repeated participation between 2000 and 2019. Table 20 compiles the overall decreases calculated by the Theil–Sen method. In all countries except Germany, the limited number of samples did not allow to determine statistically significant decreases.

The highest HCB concentrations (range 13.5 µg/kg to 31.6 µg/kg) were found in countries participating in the 2000–2003 period. The highest overall decrease over 10 years of 48% was observed in Germany, with a decrease of the median of four pooled samples collected in 2002 (31.6 µg HCB/kg lipid as median, range 28 µg/kg to 37 µg/kg) by 67% until 2019 (10.4 µg/kg as median, range 10.2 µg/kg to 10.6 µg/kg). In most other countries, in particular with lower initial HCB concentrations, the decreases were lower. Over shorter periods, the levels remained quite constant in Australia (from 2010 to 2013) or increased slightly in Norway (from 2001 to 2006).

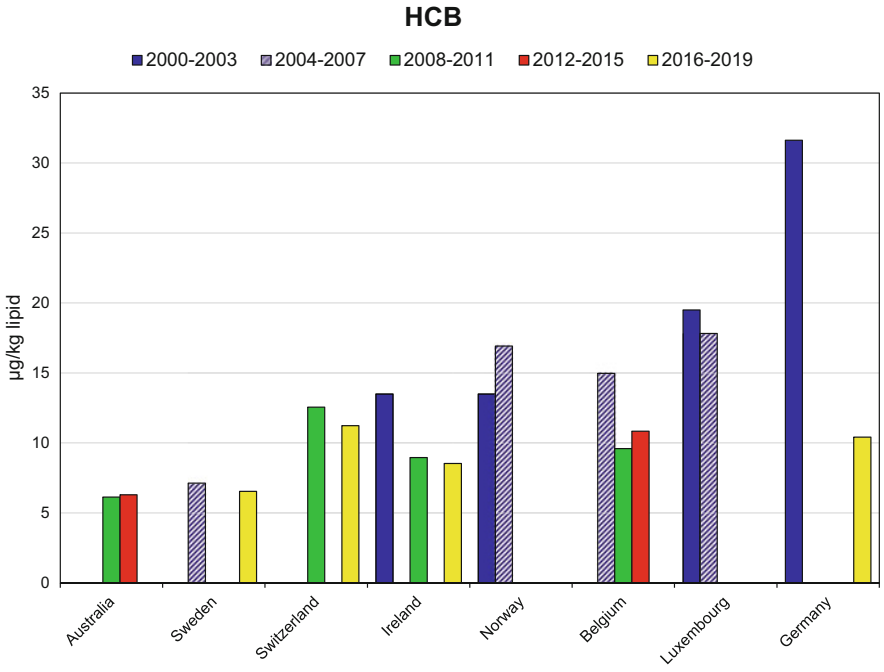

Fig. 34 Overview of the development of the HCB concentration in human milk (expressed as µg/kg lipid; aggregated data) over time in countries of the Western European and Others Group with repeated participation between 2000 and 2019

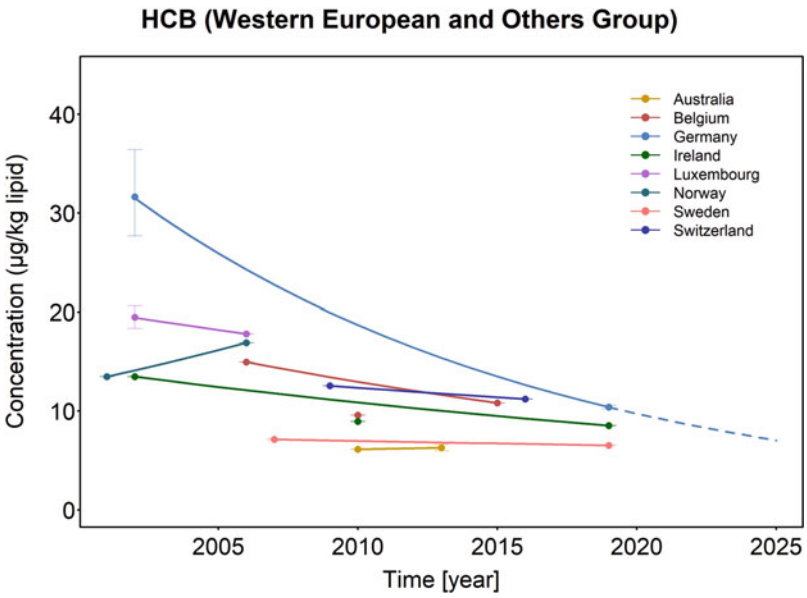

Fig. 35 Temporal tendencies of the HCB concentration in human milk (expressed as µg/kg lipid) in countries of the Western European and Others Group with repeated participation between 2000 and 2019 using the Theil–Sen method (with a statistically significant time trend in Germany)

Table 20 Overall decrease (%) of the HCB concentration in human milk (expressed as µg/kg lipid) in countries of the Western European and Others Group per 1 year, 5 years and 10 years and for one country, an estimated concentration in 2025 (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 5 years	Overall decrease (%) per 10 years	Estimated concentration in 2025 [ng/g lipid]	Trend *p*-value overall
Australia	−0.9	−4.4	−9.0		1.000
Belgium	3.5	16.5	30.2		0.500
Germany	6.3	27.8	47.9	7.04	0.008
Ireland	2.7	12.6	23.7		0.250
Luxembourg	2.2	10.4	19.8		0.500
Norway	−4.6	−25.3	−57.1		1.000
Sweden	0.7	3.5	6.9		1.000
Switzerland	1.6	7.7	14.7		1.000
Median	1.9	9.0	17.3		

5.7 Dependence of Decrease (Decrease Rate Constants) on Concentration

As explained in subsection 3.7, the decrease (*decrease rate constants*) within a 10-year period have to be seen also in context with the concentration range: A differentiation of levels above or in the range of background contamination seems to be advisable.

HCB concentrations in human milk in the 44 countries with repeated participation between 2000 and 2019 comprise a wide range between 1.6 μg/kg and 154 μg/kg. This was grouped into four ranges (<5; 5–10; 10–20; >20 μg HCB/kg lipid) to check the dependence of the decrease on the "initial" (the first measured) concentration in a country. The lower end of the frequency distribution below 5 μg HCB/kg lipid is considered as the background contamination.

Table 21 shows, that the decrease over 10 year for HCB concentrations vary in all ranges. However, for samples above 20 μg/kg, the highest median decrease over 10 years (63%) for five samples was observed, whereas the median decrease in the low background range was 13%. Note that all samples in the low background range had concentrations above LOQ (0.5 μg/kg lipid) with a range between 1.6 and 5.0 μg HCB/kg lipid. Figure 36 illustrates this variation of the decreases over 10 years in 44 countries with repeated participation between 2000 and 2019 in all concentration ranges, with a tendency to higher decreases at higher levels. It might be concluded that the large variation of the decrease for beta-HCH concentrations in the low background range (< 5 μg/kg) limits the applicability of this parameter for assessments at the country-level at this low level, but allows a more general assessment of the temporal trends of the background contamination.

Table 21 Overall decrease (%) of HCB concentrations in human milk (μg/kg lipid) over 10 years calculated by the Theil–Sen method and their dependence on the concentration range

	<5 μg/kg	5–10 μg/kg	10–20 μg/kg	>20 μg/kg
N	20	7	12	5
min	−69	−9	−57	−30
median	13	35	30	63
max	65	80	85	86

Negative decreases are to be read as increase

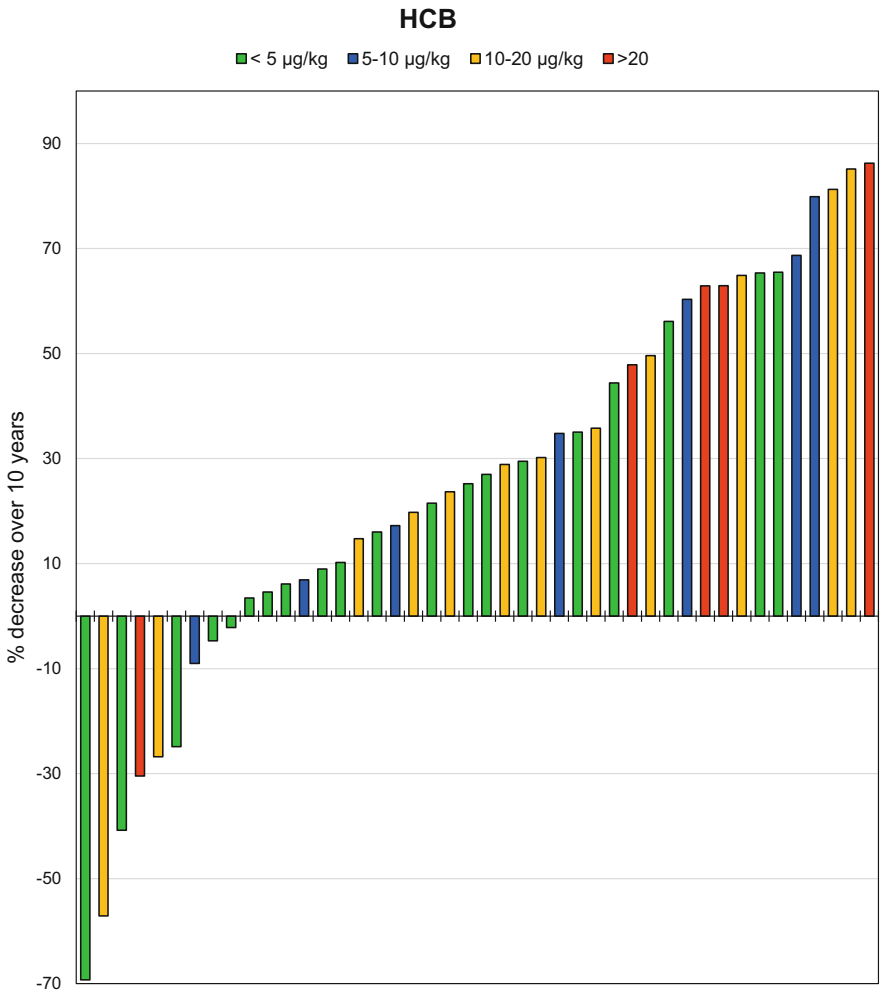

Fig. 36 Dependence of the decrease over 10 years (calculated by the Theil–Sen method) for HCB on concentrations in human milk in 44 countries with repeated participation between 2000 and 2019, with differentiation into four ranges of concentration (<5; 5–10; 10–20; >20 µg/kg lipid)

6 Summary and Conclusions

The assessment of temporal trends is an important objective of the WHO/UNEP-coordinated studies for the evaluation of the effectiveness of the Stockholm Convention on POPs to eliminate or reduce emissions of listed POPs.

The presentation and discussion of the 2000–2019 results for chlorinated pesticides and industrial chemicals in Part III of this compendium includes a *general*

estimation of time trends during the five rounds for *all* participating countries over these 20 years. However, a more precise approach is the evaluation of results from *only countries with repeated participation* in these studies: This allows more certainty in drawing of conclusions on temporal trends, which are not potentially influenced by single results of a countries submitted for a single round. The time trends of 119 pooled samples from 44 countries with repeated participation seemed optimal for the evaluation of the effectiveness for the purpose of Article 16. With regard to the found concentrations, most relevant were DDT (as sum parameter "DDT complex", comprising the p,p′-and o,p′-isomers of DDT and the metabolites p,p′-DDE and p,p′-DDD), beta-HCH and HCB, which are presented in this chapter.

For statistically significant *trends*, a minimum of five data points has to be available. However, for most countries only less than five data points are available. This prevents deriving statistically significant temporal trends in these cases. Yet, the existing data can indicate decreasing or increasing *tendencies* in POP concentrations. Furthermore, pooling of data in regions allows to derive statistically significant time trends for the UN regional groups. To minimize possible sources of variation for time trend analysis of POPs, the concept of the WHO/UNEP-coordinated exposure studies has two basic elements (preparation of pooled samples from a number of individual samples considered to be representative for a country or region/subgroup; analysis by a reference laboratory).

To provide reliable monitoring information for the Parties to the Stockholm Convention, as quantitative objective for temporal studies *The Guidance Document on the Global Monitoring Plan* (GMP) proposed the ability to detect a 50% decrease in the levels of POPs within a 10-year period. However, there is no stipulation that this is a quantitative goal for the rate of decrease in the levels of listed POPs. The Convention's objectives are either to eliminate or to reduce production, use and releases, depending on the annex where a chemical is listed, but the rate of the change is nowhere specified or required.

Decreases (*decrease rate constants*) per 1 year, 5 years (about 20% higher than for 4 years as average lengths of the WHO/UNEP-coordinated studies) and 10 years were computed using the Theil–Sen trend estimator including values of all individual samples for the trend analysis. For confirmation, a method of deriving the regional trend as a median of trends in countries within the region was used ("median method").

DDT

For DDT concentrations in human milk, large differences were found comprising a range between a minimum of 17 μg DDT complex/kg lipid found in 2019 and a maximum of 23,500 μg DDT complex/kg lipid found in 2012 (median 283/kg).

An overall decrease within a 10-year period between 50% and 80% was achieved for DDT complex levels in Africa, the Asia-Pacific Group and the Group of Latin American and Caribbean Countries, and at a global level. Lower decreases were observed in the Eastern European Group and the Western European and Others Group. Generally, the highest DDT concentrations in the five periods between 2000 and 2019 were found in the Africa Group, the Asia-Pacific Group or the Group Latin America and Caribbean Countries, whereas Western European countries had the

lowest median and lowest maximum of DDT concentrations. This is an indication that the decrease might be faster in regions with higher concentration, compared to a slower decrease in less contaminated regions, which banned DDT decades ago. This is supported by the assessment of the reduction rates based on the frequency distribution of DDT concentrations in 44 countries showing that the decrease over 10 year in the upper part of the frequency distribution for the three samples with concentrations above 2000 μg DDT complex/kg lipid is considerably higher than in the lower part (<200 μg DDT complex/kg lipid).

Beta-HCH

Large differences were also found of beta-HCH concentrations, with a minimum of <0.5 μg/kg in few countries and a maximum of 1020 μg/kg lipid in a sample of 2002 (median: 6.0 μg/kg). An overall decrease within a 10-year period between 50% and 98% was achieved for beta-HCH levels in the UN regional groups and at the global level.

On the country level, the reduction rates should also be seen in context with the concentration range (differentiation of levels above or in the range of the background contamination). If high levels are found, sources might be detected which could be eliminated. However, at low background levels (< 5 μg beta-HCH/kg lipid), other factors, e.g. contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally.

HCB

For HCB, the maximum levels and therefore the ranges were much lower than found for DDT and beta-HCH, with a minimum of about 1–2 μg/kg found in some countries and a maximum of 154 μg/kg found in 2009 in an Eastern European country (median: 6.1 μg/kg).

In the African Group with all pooled samples and all collection periods, the low background range at or below 5 μg HCB/kg lipid resulted the overall decrease within 10 years of only about 11% calculated by the Theil–Sen method. Overall decreases between 30% and 67% were calculated by the Theil–Sen method for the other UN Regional Groups, and 57% on a global level.

However, the high variation of the reduction rates in African countries with all samples at all times in the lower background range at or below 5 μg HCB/kg lipid shows that use of these calculated reduction rates is questionable in this low range. As concluded for beta-HCH, the large variation of decrease rates for HCB concentrations in the low background range (<5 μg/kg lipid) limits the applicability of this parameter for assessments at the country-level at these low levels, but allows a more general assessment of the temporal trends of the low background contamination.

Overall Conclusions

The concept of WHO/UNEP-coordinated exposure studies with standardized protocols for preparation of pooled samples considered to be representative for a country or subgroup within a country and analysis in a reference laboratory with

long-term quality control provides a cost-effective method to obtain reliable data on POPs in human milk samples over time. The use of only those countries with repeated participation provides the best possible data base for assessment of temporal trends. Statistically significant decreasing trends for DDT, beta-HCH and HCB were observed for all parameters in the five UN regional groups and at a global level. However, for the majority of individual countries the limited available data did not allow for the statistically significant assessment of time trends, but decreasing tendencies were observed for many of them and constant or increasing levels for few of them. It is highly recommended to continue this monitoring effort to secure enough data for a proper time trend assessment in the future.

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing Regional Support for the POPs Global Monitoring Plan Under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

Data analysis was supported by the RECETOX Research Infrastructure (No LM2018121) and the CETOCOEN EXCELLENCE Teaming Phase 2 project financed by the European Union's Horizon 2020 (No 857560) and the European Structural and Investment Funds: (No CZ.02.1.01/0.0/0.0/17_043/0009632).

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratory, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory and administrative staff. The continuous exchange of information between the National Coordinators and WHO, UNEP and the Reference Laboratory was an important aspect for the successful organization of these studies on a global level.

Hae Jung Yoon, Seongsoo Park and Philippe Verger (Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO, and Lawrence Grant (WHO) for the statistical analysis of the sampling protocols.

The authors thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

The authors also thank the team at CVUA Freiburg for performance of the analyses and for the scientific support and cooperation with the national coordinators.

Disclaimer The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions, policy or views of the World Health Organization and the United Nations Environment Programme.

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Time Trends in Human Milk Derived from WHO- and UNEP-Coordinated Exposure Studies, Chapter 3: Perfluoroalkyl Substances (PFAS)

Rainer Malisch, Peter Fürst, Kateřina Šebková, Daria Sapunova, and Jiří Kalina

Abstract

Temporal trends of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonic acid (PFHxS) were assessed using 86 pooled human milk samples from 59 countries from all United Nations Regional Groups collected between 2008 and 2019 as part of the WHO/UNEP-coordinated exposure studies. The primary objective of these temporal studies is to provide monitoring data for the effectiveness evaluation of the Stockholm Convention on Persistent Organic Pollutants (POPs). General temporal trends were estimated using data from all participating countries by grouping into three equal four-year periods (2008–2011, 2012–2015, and 2016–2019) reflecting the performance of three rounds of the studies. A more precise approach is the use of data from 24 countries with repeated participation in the WHO/UNEP-coordinated exposure studies, 22 of them in different periods and two in the same period. However, there were no Western European countries with multiple participation, and only two countries from the Asia-Pacific Region with one country submitting two samples in the same period.

The country-specific PFOS data showed decreasing tendencies in 19 of the 22 countries with participation in different periods, quite constant levels in two countries and increasing tendencies in one country (from its first participation in 2010 to the second participation in 2015). For the two countries with repeated

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_14

participation in the same period, it does not seem appropriate to derive country-specific temporal tendencies. An overall decrease of PFOS concentrations over 10 years of 48% and 52%, respectively, was calculated by the Theil–Sen method (1) using all samples and (2) using samples from countries with repeated participation. PFOA concentrations showed decreasing tendencies in 17 countries of the 22 countries with participation in different periods, quite constant levels in two countries and increasing tendencies in three countries (from their first participation between 2009 and 2011 to the second participation in 2015). An overall decrease over 10 years for PFOA concentrations of 42% and 47%, respectively, was calculated by the Theil–Sen method (1) using all samples and (2) using samples from countries with repeated participation. The estimation of general temporal trends for PFHxS was not possible, as in 84% of the samples PFHxS concentrations were below the limit of quantification (5.7 ng/L).

Keywords

Time trends · Human milk biomonitoring · Stockholm Convention on Persistent Organic Pollutants · Perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), Perfluorohexane sulfonic acid (PFHxS) · Global WHO/UNEP studies · UN Regional Groups

1 Introduction

Between 2000 and 2019, the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) performed five global studies on concentrations of POPs in human milk (Malisch et al. 2023a). The number of analytes in these studies gradually expanded as new POPs were listed in the annexes of the Stockholm Convention, including perfluorooctane sulfonic acid (PFOS) (UNEP 2009; UNEP 2019a), perfluorooctanoic acid (PFOA) (UNEP 2019b), and perfluorohexane sulfonic acid (PFHxS) (UNEP 2019c; UNEP 2022). PFOS, PFOA, and PFHxS were included as targeted compound in samples of the studies collected between 2008 and 2019 and analysed at the Örebro University, Örebro, Sweden.

Data on the regional occurrence of these substances during 2016–2019 comprise 44 human milk samples collected in 42 countries. PFOS was quantifiable in 36 samples across a wide range (total PFOS between <6.2 pg/g and 212 pg/g, calculated as sum of linear PFOS [L-PFOS] and branched isomers [br-PFOS]); PFOA was quantified in all 44 samples in a narrower range (6.20 pg/g–37.4 pg/g); PFHxS was quantifiable in only four samples (max. 111 pg/g). Branched PFOS isomers on average had a share of 16% of the total PFOS with a maximum of 33% (Fiedler and Sadia 2021).

Data on 101 samples consisting of 86 national pools and 15 pools from States in Brazil obtained between 2008 and 2019 were used to estimate temporal trends. It was explained that the GMP guidance (UNEP 2019d) stipulates the goal to achieve 50% reduction of POPs concentrations in the core matrices over a 10-year period. It

was concluded that the Stockholm Convention goal of 50% reduction in 10 years was achieved for PFOS by Antigua and Barbuda, Kenya, and Nigeria and for PFOA by Antigua and Barbuda, only. In a few cases, increases were observed; in one country for PFOS, in four countries for PFOA (Fiedler et al. 2022).

In Part IV of this compendium on WHO/UNEP-coordinated exposure studies on human milk, three specific articles assess time trends. Two approaches can be used: (1) temporal trends derived from all participating countries; (2) temporal trends derived only from countries with multiple participation in WHO/UNEP-coordinated exposure studies. The first article "Time Trends in Human Milk Derived from WHO- and UNEP-Coordinated Exposure Studies, Chapter 1" introduced various aspects and presented results for polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) (Malisch et al. 2023b), the second article (Chapter 2) on DDT [dichlorodiphenyltrichloroethane], beta-hexachlorocyclohexane (beta-HCH), and hexachlorobenzene (HCB) (Malisch et al. 2023c). In addition, temporal trends for polybrominated diphenyl ethers (PBDE) were assessed as part of the presentation of findings of polybrominated substances (Schächtele et al. 2023). This article (as Chapter 3 of the three specific articles on time trends) presents the assessment of temporal trends of perfluoroalkyl substances (PFAS).

2 Material and Methods

2.1 Source of Data

Per- and polyfluoroalkyl substances (PFAS) were analysed at the Örebro University, Örebro, Sweden and the analytical methods and results published (Sadia et al. 2020; Fiedler and Sadia 2021; Fiedler et al. 2022). The results were presented on product basis (as pg/g fresh weight). *(PFAS have different physical and chemical properties than the chlorinated and brominated POPs listed by the Convention, which are reported on lipid basis.)*

The data belong to UNEP and the sample-submitting countries and are publicly available in the Data Warehouse of the Stockholm Convention Global Monitoring Plan (GMP DWH) (GMP DWH 2020). For this article, the data base of the GMP DWH was used providing PFAS data on volume basis (as ng/L). Data of 86 pooled samples obtained from 59 countries between 2008 and 2019 were used.

2.2 Methods of Statistical Data Treatment: Trends vs. Tendencies

For methods of statistical data treatment, see subsection 2.4 in the preceding article "Time Trends in Human Milk Derived from WHO- and UNEP-Coordinated Exposure Studies, Chapter 1" on time trends for PCB, PCDD, and PCDF (Malisch et al. 2023b). This included the non-parametric linear Theil–Sen trend estimator and the median method to derive decreases (*decrease rate constants*) that are expected to

show exponential trends (as commonly observed in cases after stop of production and application of a chemical rather than unrealistic linear trends). Non-detects were substituted by 0.707 multiple of the detection limit.

We differentiate between *trends* as statistically significant decrease (requiring *p*-values <0.05) and changes of concentrations indicating *tendencies* as statistically not significant decrease. Simulations show that Theil–Sen p is never below 0.05 for fewer than 5 data points. As for all countries with repeated participation only two data points were available, the observed changes of PFAS concentrations in these countries are statistically not significant and therefore only indicate tendencies. However, on regional and global basis, data from countries with repeated participation can be combined to provide more than 5 data points allowing to derive statistically significant trends.

The median method is based on tendencies of individual countries. Thus, the inclusion of countries in the median method is not possible, if a country did not participate at least twice. Therefore, the results of the median method applied for the UN regional groups comprising all countries are the same as for the UN regional groups comprising only countries with repeated participation. However, the results of the worldwide median method are calculated as median trend of the five regional trends and not based on individual countries trends. Therefore, the results of the worldwide median method computed for PFAS concentrations using all countries are not the same as using only countries with repeated participation.

As PFOS was listed under the Stockholm Convention already in 2009, PFOA only in 2019, and PFHxS was through the assessment procedure but not yet through the process of adoption, the Hites method of break point search was used to check whether a break point between possibly increasing and decreasing trends in the samples collected between 2009 and 2019 could be found (Hites 2019).

3 Results and Discussion

The assessment of temporal trends is a key element for the effectiveness evaluation of the Stockholm Convention on Persistent Organic Pollutants (POPs) and human milk is a core matrix for this purpose. As the first step, time trends can be derived based on data from all participating countries. However, levels among countries are often highly variable. Therefore, a more precise approach is the assessment of temporal trends by considering only countries with repeated participation in the WHO/UNEP-coordinated exposure studies. Because levels correlate within countries more closely than among countries, this allows more certainty in drawing conclusions on time trends which are not potentially influenced by individual results from countries submitted just for a single round and seems optimal for the evaluation of the effectiveness for the purpose of Article 16 of the Convention. However, it should be noted that typically only very few time points from most individual countries are available, which prevents from deriving statistically significant temporal trends in these cases. Yet, the existing data can still indicate decreasing or increasing tendencies in POP concentrations. Nevertheless, pooling data in regions

allows to derive statistically significant time trends in the UN regional groups and globally.

To provide reliable monitoring information for the Parties to the Stockholm Convention, the GMP guidance document proposed a quantitative objective for temporal studies: These studies should be able to detect a 50% decrease in the levels of POPs within a 10-year period (UNEP 2015; UNEP 2019d). However, in distinction from this goal for abilities of temporal studies to detect changes over time, there is no stipulation of a quantitative goal for the rate of reduction/decrease in POPs levels, e.g. in the core matrices by the GMP guidance document or by the Convention. The Convention's objectives are either to eliminate or to reduce production, use, and releases, depending on the annex where a chemical is listed, but the rate of decline is nowhere specified or required.

For the evaluation of time trends, the 86 pooled samples obtained from 59 countries between 2008 and 2019 can be grouped, e.g. into three 5-year periods: 2005–2009, 2010–2014, 2015–2019 (Fiedler et al. 2022). However, the samples were obtained between 2008 and 2019, with 3 samples from 2008 and 14 samples from 2009. Thus, the first period (2005–2009) is rather a one- or two-year period comprising the years 2008 and mainly 2009. Three equal four-year periods, e.g. 2008–2011, 2012–2015, and 2016–2019 reflect more closely the performance of rounds in the WHO/UNEP-coordinated exposure studies (fifth, sixth, and seventh survey) (Malisch et al. 2023a), seem to be more appropriate and were used in this article.

The regional distribution of samples and the regional distribution within each of the three periods varied considerably, with most samples originating from the African region. There was no sample from Western European and Others Group (WEOG) in the first period (2008–2011). The second period (2012–2015) has the lowest number of samples in all UN Regions except the Eastern European Group (EEG) (Table 1). Note that two countries submitted two samples in different years of the same period: Chile (2008, 2011) and Niue (2017, 2019; the sample of 2017 was sent for analysis of all POPs and the sample of 2019 only for determination of PFAS). Furthermore, Germany submitted two sub-pools in 2019.

Table 1 Regional distribution of national pools and their distribution within each of three periods

	Countries	Number of national samples			
	(all)	2008–2011	2012–2015	2016–2019	Total
Africa	16	11	2	14	27
Asia-Pacific[a]	16	5	0	13	18
GRULAC[b]	12	8	4	9	21
EEG	8	3	7	2	12
WEOG[c]	7	0	2	6	8
Sum	59	27	15	44	86

[a]1 Country with two samples in the same period (Niue, 2017 and 2019)
[b]1 Country with two samples in the same period (Chile, 2008 and 2011)
[c]1 Country with two sub-pools (Germany, 2019)

Table 2 Regional distribution of national pools obtained from countries with repeated participation and their distribution within each of three periods

	Countries	Number of national samples (repeated participation)			
	(repeated participation)	2008–2011	2012–2015	2016–2019	Total
Africa	11	11	2	9	22
Asia-Pacific[a]	2	1	0	3	4
GRULAC[b]	7	8	1	5	14
EEG	4	3	4	1	8
WEOG	0	0	0	0	0
Sum	24	23	7	18	48

[a]1 country with two samples in the same period (Niue, 2017 and 2019)
[b]1 country with two samples in the same period (Chile, 2008 and 2011)

Table 3 Regional distribution of years between samplings

	Countries with repeated participation	Countries with data for 2008–2011 and 2016–2019	Years between samplings	Countries with data for two subsequent periods	Years between samplings	Countries with data for the same period	Years between samplings
Africa	11	9	7–11	2	4–5	0	–
Asia-Pacific	2	1	8	0	–	1	2
GRULAC	7	5	6–10	1	4	1	3
EEG	4	0	–	4	5–6	0	–
WEOG	0	0	–	0	–	0	–
Sum	24	15		7		2	

24 countries participated repeatedly between 2008 and 2019 submitting in total 48 samples (Table 2). 22 of these countries submitted samples in different periods, two countries in the same period (Chile, 2008 and 2011, and Niue, 2017 and 2019). Most samples came from African countries ($N = 22$), followed by the Group of Latin America and the Caribbean Countries (GRULAC) ($N = 14$). There were no WEOG countries with repeated participation in these three periods.

With regard to the length of the time-series, the GMP guidance document considers it naïve to expect monitoring of POPs to reveal temporal trends with any confidence within a sampling period of five years unless the changes are very large. More suitable would be a period of at least 10–15 years to detect significant changes of moderate size (5% /year) (UNEP 2019d). Table 3 gives an overview of the number of years between the samplings in the UN regions. For 15 countries, PFAS results are available for the first (2008–2011) and third (2016–2019) period with 6–11 years between samplings. In 7 countries, data are available for two subsequent periods (either [i] 2008–2011 and 2012–2015 or [ii] 2012-–2015 and 2016–2019) with 4–6 years between samplings. In two countries, samples were collected in the same period, with 2–3 years between samplings.

3.1 PFOS

PFOS was listed in Annex B of the Convention in 2009 (UNEP 2009).

3.1.1 Estimation of Temporal Trends by Differentiation into Three Periods between 2008 and 2019

As PFAS analysis was initiated with samples submitted in 2008, time trends for PFOS can be derived beginning with the 2008–2011 period. As a starting point, descriptive parameters summarizing the results of 86 national pools from 59 countries were used as basis for the first *general* estimation of temporal trends with differentiation into three equal four-year periods between 2008 and 2019. The median of the PFOS concentrations increased from 24.6 ng/L in the 2008–2011 period ($N = 27$) to 36.0 ng/L in the 2012–2015 period ($N = 15$) and then decreased to 13.6 ng/L in the 2016–2019 period ($N = 44$). The highest maximum level (218 ng/L) was found in the 2016–2019 period (Fig. 1).

Thus, the summarizing descriptive parameters seem to indicate an increase of the PFOS concentrations after listing in 2009 until the 2012–2015 period. However, it has to be checked whether these fluctuations of the median are likely due to the result of participation of different countries in different rounds of the WHO/UNEP-coordinated exposure studies. Therefore, as described for PCDD/PCDF, PCB, DDT, beta-HCH, and HCB in the first two chapters of the three specific articles on assessment of time trends, also for PFOS a more precise evaluation based on country-specific results is necessary.

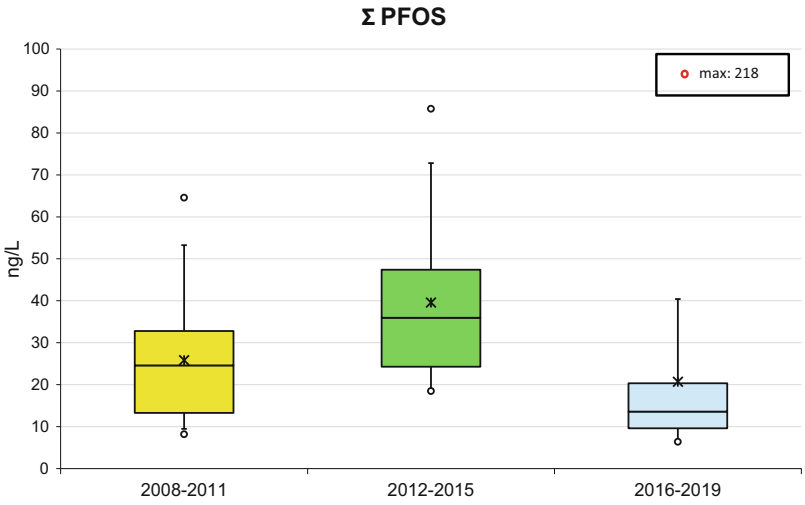

Fig. 1 Range of ∑PFOS concentrations (ng/L) in all samples over three periods ($N = 27$ in 2008–2011; $N = 15$ in 2012–2015; $N = 44$ in 2016–2019) [box plot; minimum and maximum: as circles; fifth and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

3.1.2 African Group

In the 27 national pools from 16 African countries, ∑PFOS concentrations between 2008 and 2019 ranged <6.4–45.9 ng/L, with downward tendencies from the 2008–2011 period to 2016–2019 in 9 countries with repeated participation (decrease by 44% as median of the observed decrease between the first and second submission [without normalization, e.g. to a 10-year period]), with quite constant concentrations between 2011 and 2015 in Niger and increasing concentrations from 2010 (33.3 ng/L) to 2015 (45.9 ng/L) in Côte d'Ivoire (Fig. 2).

Figure 3 illustrates the temporal tendencies in countries with multiple participation using the Theil–Sen method (comprising all individual pooled samples of countries with repeated participation and assuming exponential trends, see subsection 2.2). The overall decrease per 1 year and 10 years is given in Table 4. The limited number of samples did not allow to determine a statistically significant decrease for countries ($p \sim 1.000$) (for statistical significance of *trends* requiring p-values <0.05 and changes of concentrations indicating *tendencies*, see subsection 2.2). As median of 11 countries, the levels of ∑PFOS in all African countries decreased within a 10-year period by 48%. This is in line with the statistically significant ($p < 0.001$) decrease over 10 years of 50.3% for this UN region derived by countries with multiple participation and calculated by the Theil–Sen method (see Table 5 in the following).

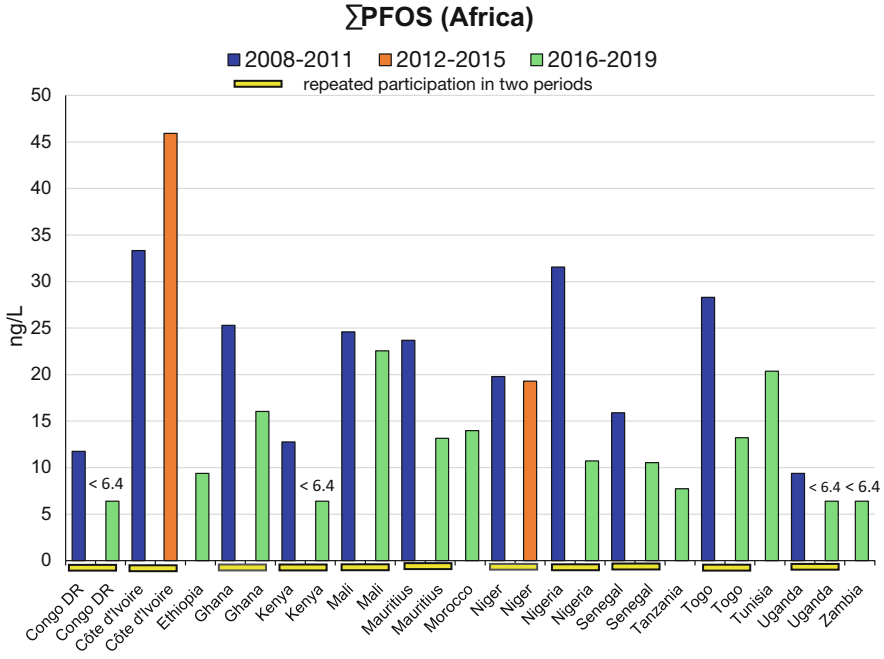

Fig. 2 Overview of the development of ∑PFOS concentrations in human milk (ng ∑PFOS/L) from African countries over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles)

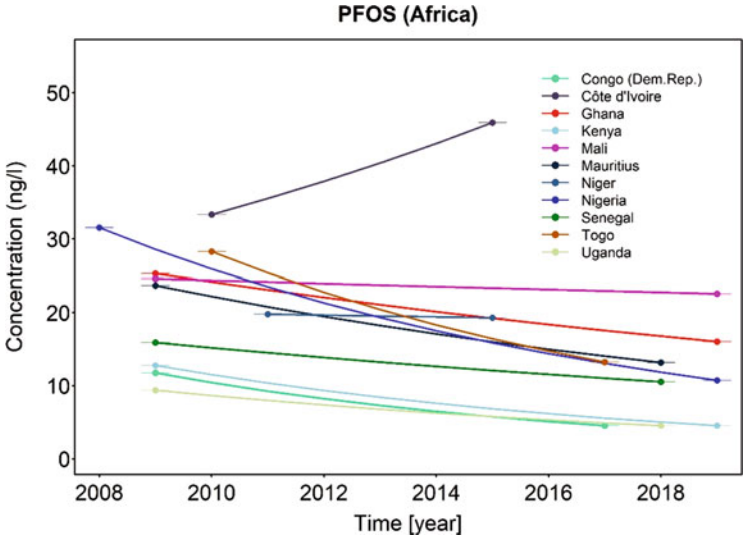

Fig. 3 Temporal tendencies of ∑PFOS concentrations (ng ∑PFOS/L) for African countries with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 4 Overall decrease (%) of ∑PFOS concentrations per 1 year and 10 years in African countries (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Congo (DR)	11.2	69.7	1.000
Côte d'Ivoire	−6.6	−89.7	1.000
Ghana	4.5	36.6	1.000
Kenya	9.9	64.6	1.000
Mali	0.9	8.3	1.000
Mauritius	6.3	48.0	1.000
Niger	0.7	6.3	1.000
Nigeria	9.3	62.5	1.000
Senegal	4.5	36.7	1.000
Togo	10.3	66.3	1.000
Uganda	7.8	55.5	1.000
Median	6.3	48.0	

Statistically significant time trends can be derived in the UN regional groups by pooling of data from countries. The time trends of ∑PFOS concentrations in the African region derived by the Theil–Sen method are illustrated by Fig. 4 for all 27 national pools from 16 countries and by Fig. 5 for 22 national pools from 11 countries with multiple participation. An overall decrease over 10 years for

Table 5 Overall decrease (%) of PFOS concentrations in the African Group computed (1) using all samples and (2) using samples from countries with repeated participation

African Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	16	6.8	6.3	50.3	48.0	<0.001
Repeatedly	11	6.4	6.3	48.5	48.0	≤0.001

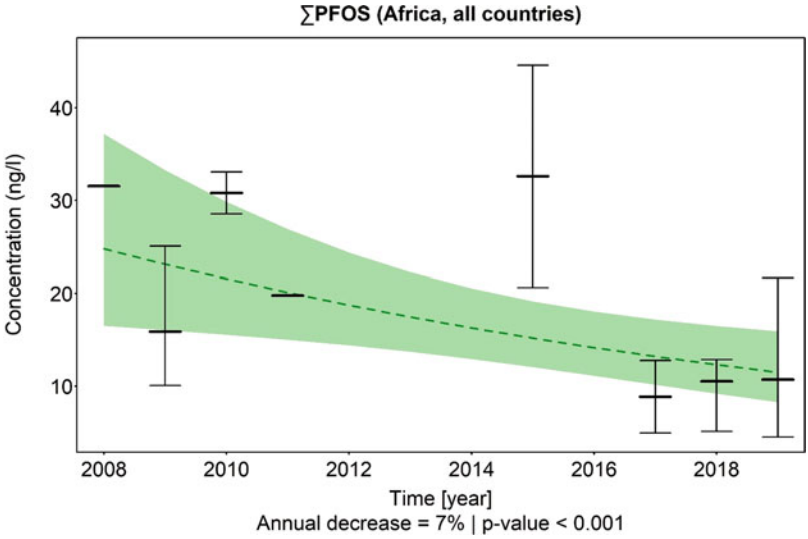

Fig. 4 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in all African countries (27 national pools, 16 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

PFOS concentrations in the African Group of 50.3% and 48.5%, respectively, was determined by using (1) all samples and (2) samples from countries with repeated participation (Table 5).

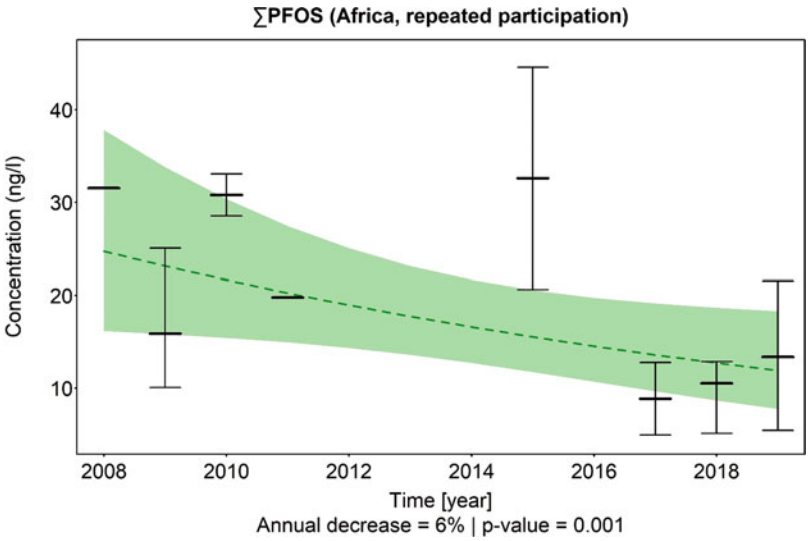

Fig. 5 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in African countries with repeated participation (22 national pools, 11 countries)

3.1.3 Asia-Pacific Group

In the most recent period (2016–2019), the pooled samples from 12 countries had ∑PFOS concentrations in the range < 6.4–30 ng/L (median 17.7 ng/L); however, the ∑PFOS concentrations in the sample from Kiribati exceeded this range by an order of magnitude (218 ng/L). For comparison, in the period 2008–2011, ∑PFOS concentrations were in the range 8.2–27 ng/L (median 13.8 ng/L; $N = 5$); however, different countries participated in these two periods. From the 16 countries of the Asia-Pacific region, data covering two periods were available only for Fiji showing a reduction of 76% from 2011 (27 ng/L) to 2019 (<6.4 ng/L) (Fig. 6).

Figure 7 illustrates the temporal tendencies observed in the two countries with multiple participation using the Theil–Sen method (comprising the individual pooled samples and assuming exponential trends, see subsection 2.2). The limited number of samples did not allow to determine a statistically significant decrease for Fiji ($p \sim 1.000$) (Table 6). The short period between the two samplings in Niue (2017 and 2019) should not be used for calculation of temporal trends over a 10 year-period.

Temporal trends of ∑PFOS concentrations in the Asia-Pacific region were assessed by the Theil–Sen method combining all 18 national pools from 16 countries (Fig. 8). As explained above, regional trends cannot be derived based on countries with multiple participation due to lack of a sufficient number of countries. Overall rates for changes over time were computed by the Theil–Sen method using all samples and showed a statistically not significant increase of 25% over 10 years

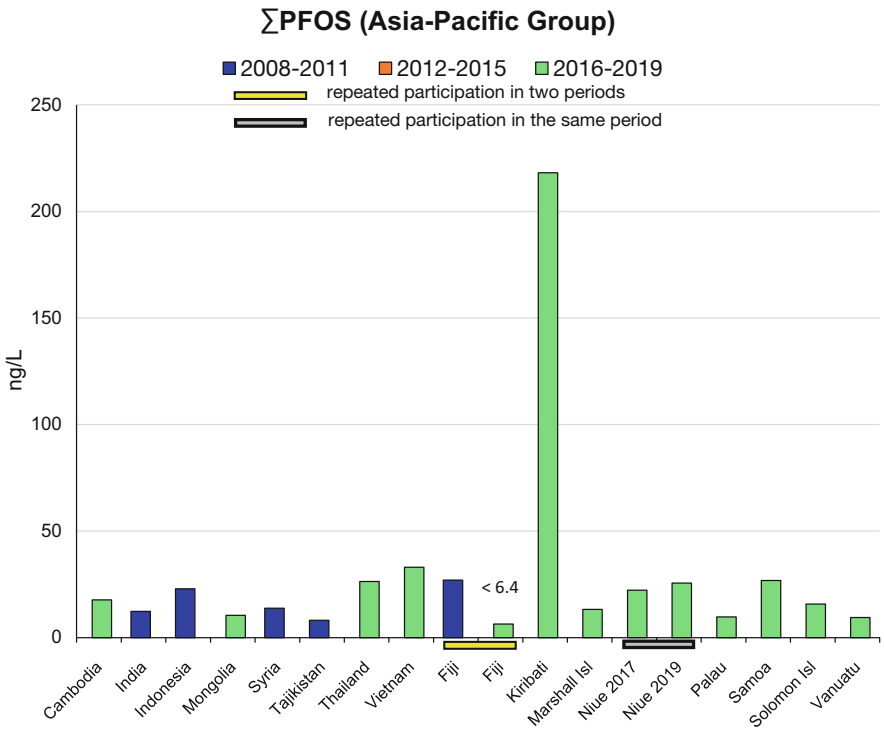

Fig. 6 Overview of the development of ∑PFOS concentrations in human milk (ng ∑PFOS/L) from Asia-Pacific countries over time (period 2008–2011 in blue, period 2012–2015 in orange [however, no samples in this period], and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles, with repeated participation in the same period by grey rectangles)

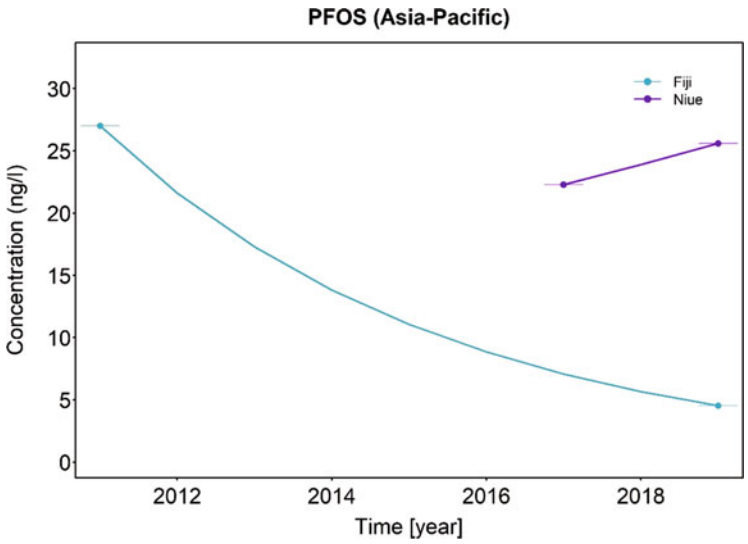

Fig. 7 Temporal tendencies of ∑PFOS concentrations (ng ∑PFOS/L) for countries of the Asia-Pacific Group with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 6 Overall decrease (%) of ∑PFOS concentrations per 1 year and 10 years in human milk from Fiji (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Fiji	20.0	89.3	1.000

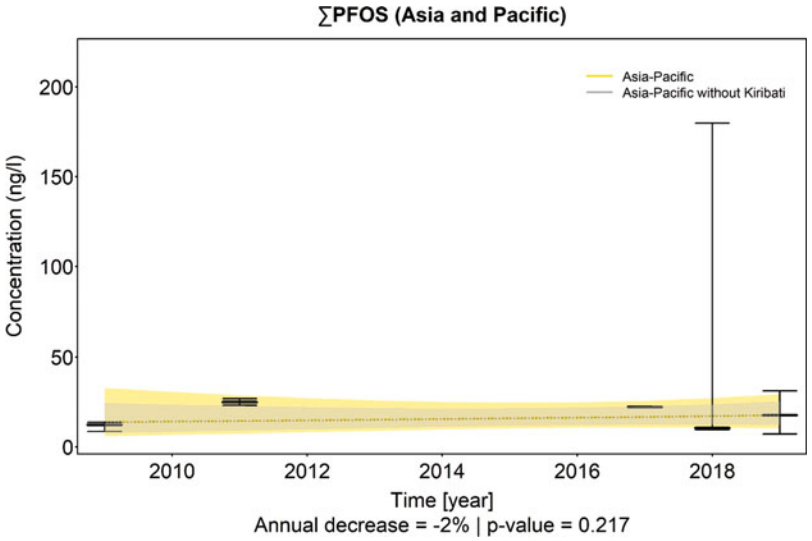

Fig. 8 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in countries of the Asia-Pacific group (18 national pools, 16 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

Table 7 Overall decrease (%) of PFOS concentrations in the Asia-Pacific Group computed using all samples (n.a. = not applicable). Negative decreases are to be read as increase

Asia-Pacific Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All samples	16	−2.3	n.a.	−24.9	n.a.	0.217

(Table 7). Only two countries (Fiji and Niue) provided more than one value, however, the short period between the two samplings in Niue (2017 and 2019) should not be used for calculation of temporal trends over a 10 year-period. Therefore, in the Asia-Pacific Group the median method cannot be applied.

3.1.4 Group of Latin American and Caribbean Countries (GRULAC)

In 21 national pools from 12 GRULAC countries, ∑PFOS concentrations between 2008 and 2019 were in a range < 6.4–67.3 ng/L (maximum in one of three samples from Brazil, 2012), with downward tendencies in all 6 countries with repeated participation in different periods. In the five countries with participation in the 2008–2011 and 2016–2019 periods (Antigua-Barbuda, Jamaica, Mexico, Peru, and Uruguay), ∑PFOS concentrations decreased by 54% as median of the observed decreases between the first and second submission (without normalization, e.g. to a 10-year period). In Haiti as country with participation in two subsequent periods (2008–2011 and 2012–2015), ∑PFOS concentrations decreased by 45% from 2011 to 2015 (Fig. 9). The two samples from Chile were submitted in the same period. With regard to the short period between the collection of samples (2008 and 2011) and to observations discussed above (related to Table 1), it does not seem to be appropriate to derive temporal tendencies from these two samples.

Overall, levels decreased from 32.7 ng/L as median (range 9.6–58.6 ng/L; $N = 8$) in 2008–2011 to 12.2 ng/L as median (range < 6.4–41.7 ng/L; $N = 9$) in 2016–2019, with a higher median of 37.9 ng/L in the 2012–2015 period (range 21.7 to 67.3 ng/L;

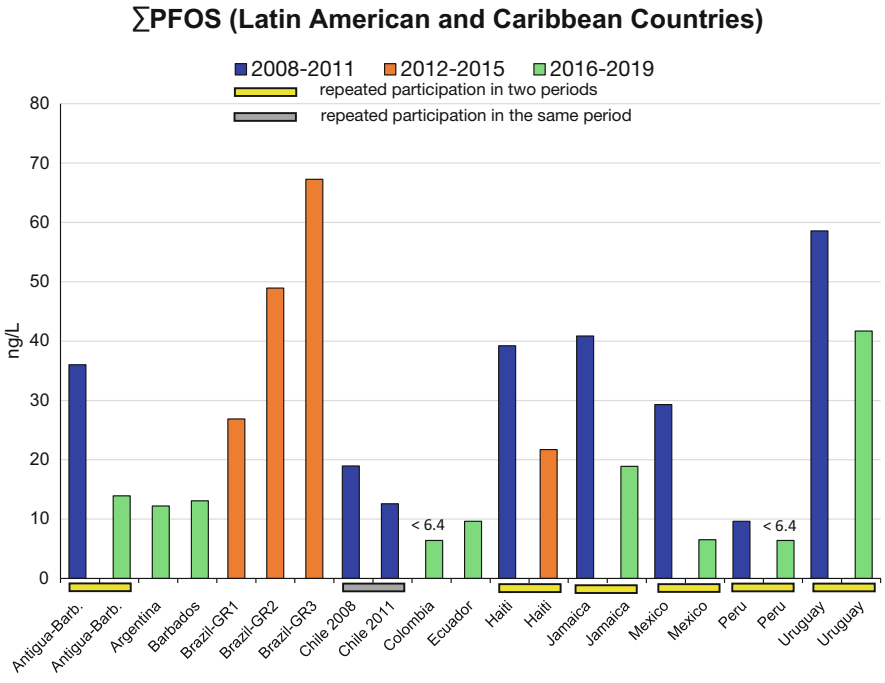

Fig. 9 Overview of the development of ∑PFOS concentrations in human milk (ng ∑PFOS/L) from countries of the Group of Latin America and the Caribbean over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods are marked by yellow rectangles, with repeated participation in the same period by grey rectangles)

$N = 4$). The observed increase from 2008–2011 to 2012–2015 demonstrates the limitations of this first *general* estimation of temporal trends, if different countries are included in different periods: The four data for concentrations in 2012–2015 are dominated by three results from one country (Brazil). Whereas in Haiti a downward tendency was observed from 2011 to 2015, temporal tendencies cannot be derived for Brazil, as only data for three samples of 2012 are available. Thus, this UN region is an example that in contrast to a general estimation of time trends from all participating countries for a region, the assessment of temporal trends from countries with repeated participation is a more precise approach because levels among countries are often highly variable and single sample contributions have a significant effect on the regional results in a certain period.

Figure 10 illustrates the temporal tendencies in countries with multiple participation using the Theil–Sen method (comprising all individual pooled samples and assuming exponential trends, see subsection 2.2). The overall decreases per 1 year and 10 years are given in Table 8. The limited number of samples did not allow to determine statistically a significant decrease for countries ($p \sim 1.000$). As median of the six countries with repeated participation in different periods, the levels of ∑PFOS in all Latin American and Caribbean countries decreased within a 10-year period by 64%. This is in line with the statistically significant ($p < 0.001$) decrease over 10 years of 66% for this UN region derived by the Theil–Sen method (see Table 9 in the following).

Time trends of ∑PFOS concentrations in this region derived by the Theil–Sen method are illustrated by Fig. 11 for all 21 national pools from 12 countries and by Fig. 12 for 12 national pools from 6 countries with multiple participation. Statistically significant decreases over 10 years of 70% and 66%, respectively, were

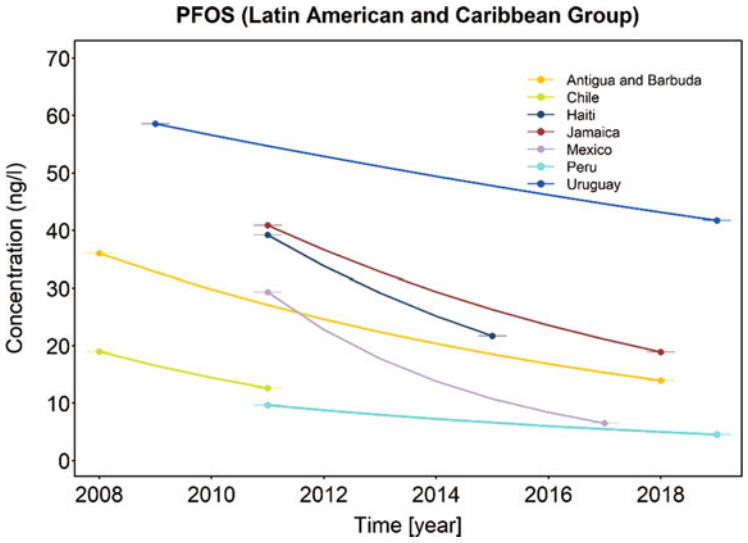

Fig. 10 Temporal tendencies of ∑PFOS concentrations (ng ∑PFOS/L) for countries of the Group of Latin America and the Caribbean with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 8 Overall decrease (%) of ∑PFOS concentrations per 1 year and 10 years in countries of the Group of Latin America and the Caribbean (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Antigua-Barb.	9.1	61.3	1.000
Haiti	13.7	77.2	1.000
Jamaica	10.4	66.8	1.000
Mexico	22.2	91.8	1.000
Peru	9.0	61.2	1.000
Uruguay	3.3	28.8	1.000
Median	9.7	64.2	

Table 9 Overall decrease (%) of PFOS concentrations in countries of the Group of Latin America and the Caribbean computed (i) using all samples and (ii) using samples from countries with repeated participation in different periods

Latin America and Caribbean	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	12	11.3	10.4	69.8	64.2	<0.001
Repeatedly	6	10.0	9.8	66.0	64.2	0.003

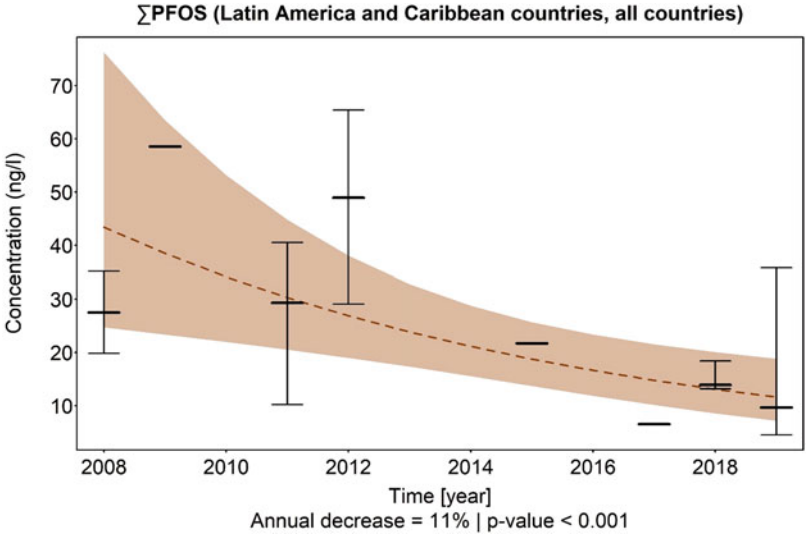

Fig. 11 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in countries of the Group of Latin America and the Caribbean (21 national pools, 12 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

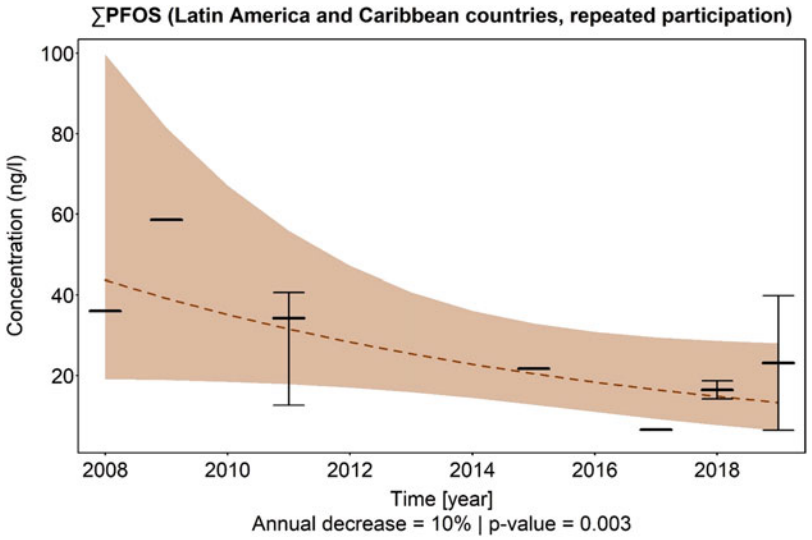

Fig. 12 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in countries of the Group of Latin America and the Caribbean with repeated participation in different periods (12 national pools, 6 countries)

achieved computed (1) using all samples and (2) using samples from countries with repeated participation (Table 9).

3.1.5 Eastern European Group

In 12 national pools from 8 Eastern European countries, ∑PFOS concentrations between 2008 and 2019 were in the range 12.6–85.5 ng/L, with constant concentrations in one country with multiple participation (Moldova, 2009 and 2015) and downward tendencies in 3 countries. In the Czech Republic, ∑PFOS concentrations decreased by 58% from 2014 to 2019, in Georgia by 39% from 2009 to 2014, and in Lithuania by 47% from 2009 to 2015 (calculated as observed decrease rates between the first and second submission [without normalization, e.g. to a 10-year period]) (Fig. 13).

Based on a quite low number of countries, overall levels decreased from 35.0 ng/L as median ($N = 3$; range 32.2–64.6 ng/L) in 2008–2011 to 13.9 ng/L as median ($N = 2$; range 12.6–15.3 ng/L) in 2016–2019, with a slightly higher median of 36.0 ng/L and a higher maximum concentration ($N = 7$; range 18.5 to 85.8 ng/L) in the 2012–2015 period. As discussed above for the Latin American and Caribbean countries, again these obviously (slightly) increasing tendencies from 2008–2011 to 2012–2015 demonstrate the limitations of this first *general* estimation of temporal trends, if different countries are included in different periods: Three countries (Bulgaria, Croatia, and Romania) are included with single participation only in the 2012–2015 period and ∑PFOS concentrations between 29.4 and 85.8 ng/L (mean:

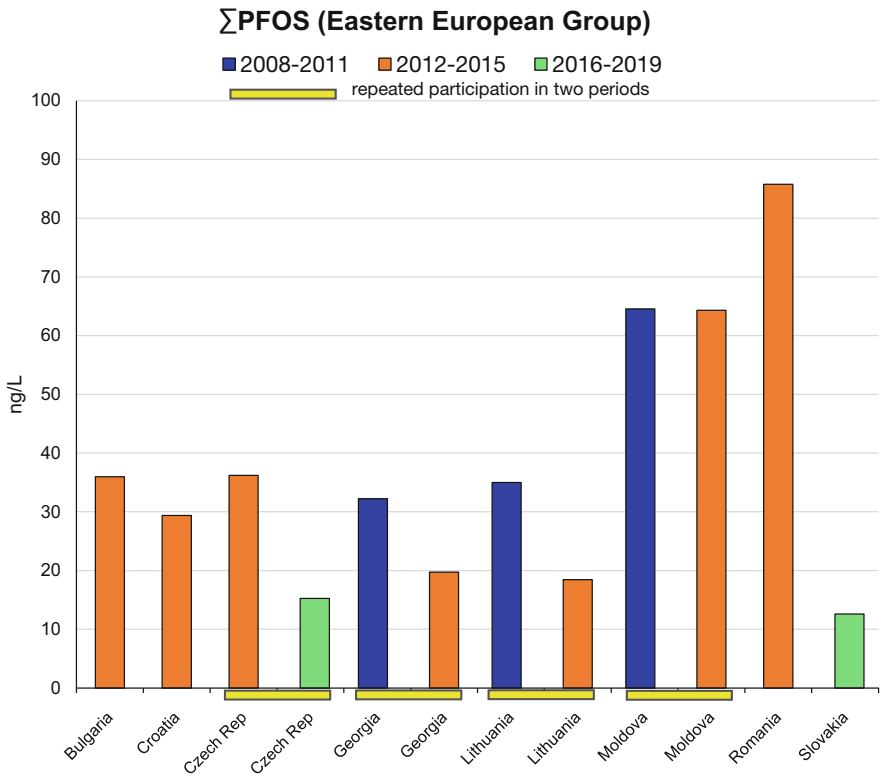

Fig. 13 Overview of the development of ∑PFOS concentrations in human milk (ng ∑PFOS/L) from countries of the Eastern European Group over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles)

50.4 ng/L). This range is higher than for the four countries with multiple participation in the 2012–2015 period (range 18.5–64.3 ng/L, mean: 34.7 ng/L).

Thus, also this UN region is an example that levels among countries are often highly variable and single contributions might have a significant effect on the regional results in a certain period, whereas temporal trends can be derived more precisely on countries with multiple participation.

Figure 14 illustrates the temporal tendencies in the four countries with multiple participation using the Theil–Sen method (comprising all individual pooled samples and assuming exponential trends, see subsection 2.2). Overall decreases per 1 year and 10 years are given in Table 10. The limited number of samples did not allow to determine statistically significant decreases for countries ($p \sim 1.000$). As median of the four countries, the levels of ∑PFOS in these Eastern European countries decreased within a 10-year period by 64%. This is in line with the statistically significant ($p < 0.001$) decrease over 10 years of 56% for this UN region derived from countries with multiple participation and calculated by the Theil–Sen method (see Table 11 in the following).

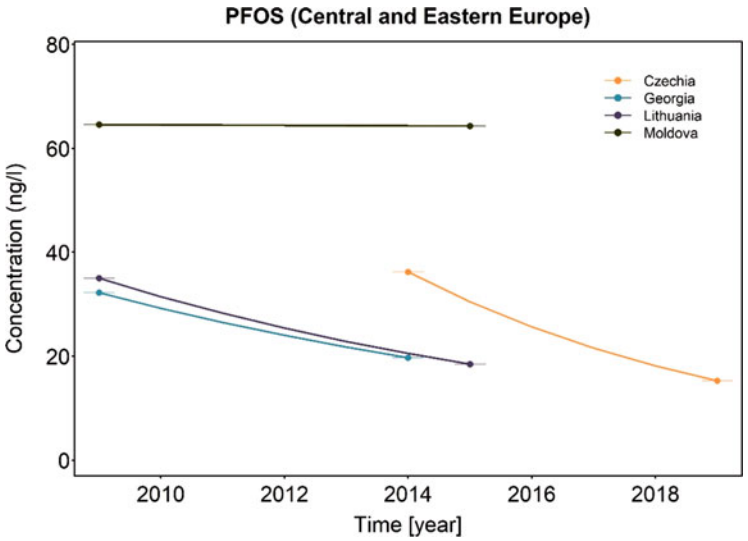

Fig. 14 Temporal tendencies of ∑PFOS concentrations (ng ∑PFOS/L) for countries of the Eastern European Group with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 10 Overall decrease (%) of ∑PFOS concentrations per 1 year and 10 years in countries of the Eastern European Group (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Czechia	15.9	82.2	1.000
Georgia	9.4	62.6	1.000
Lithuania	10.1	65.6	1.000
Moldova	0.1	0.7	1.000
Median	9.7	64.1	

Table 11 Overall decrease (%) of PFOS concentrations in the Eastern European Group (EEG) computed (1) using all samples and (2) using samples from countries with repeated participation

Eastern European Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	8	9.4	9.7	62.6	64.1	0.002
Repeatedly	4	8	9.7	56.4	64.1	0.052

Statistically significant time trends can be derived in this UN regional group by pooling of data from countries. The time trends of ∑PFOS concentrations derived by the Theil–Sen method are illustrated by Fig. 15 for all 12 national pools from 8 countries and by Fig. 16 for the 8 national pools from 4 countries with multiple

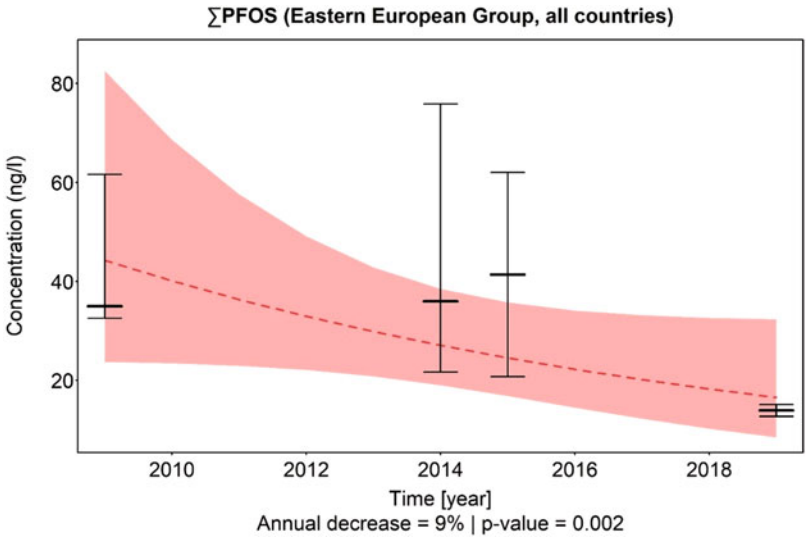

Fig. 15 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in countries of the Eastern European Group (12 national pools, 8 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

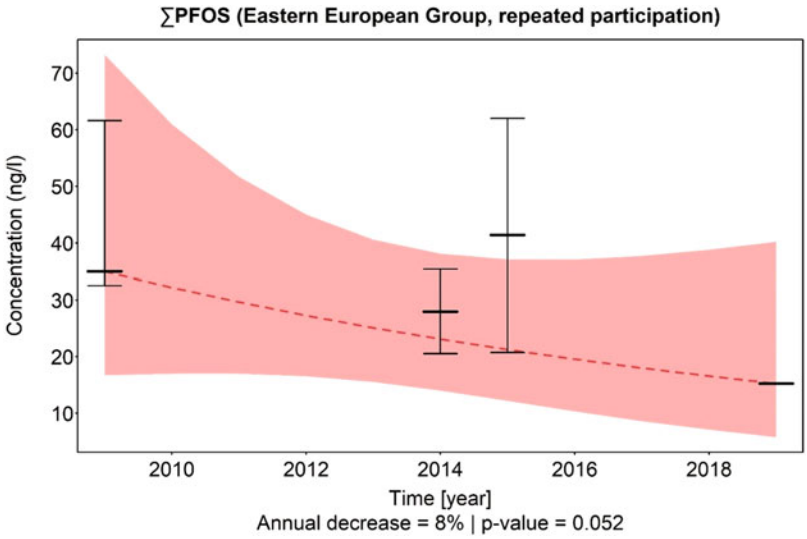

Fig. 16 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in countries of the Eastern European Group with repeated participation (8 national pools, 4 countries)

participation. Overall decreases over 10 years for PFOS concentrations of 62.6% and 56.4%, respectively, were calculated (1) using all samples and (2) using samples from countries with repeated participation (Table 11).

Although the significance of both trend estimates is relatively high (p-value 0.002 and 0.052, respectively), changes in trend slopes may be expected in the period 2011–2015 after listing of PFOS in 2009 in 2009 in Annex B of the Convention. Using the Hites method of break point search (Hites 2019) on all countries in the Eastern European Group, a break point was found for PFOS, although, the result was non-significant.

3.1.6 Western European and Others Group (WEOG)

In 8 national pools from 7 Western European countries, ∑PFOS concentrations between 2014 and 2019 were in the range 13.9–52.9 ng/L. There were no countries with multiple participation (Fig. 17). Statistically significant time trends can be derived in this UN regional group by pooling of all data from countries. The time trends derived by the Theil–Sen method are illustrated by Fig. 18. An overall decrease over 10 years of 79.1% was computed using all samples (Table 12).

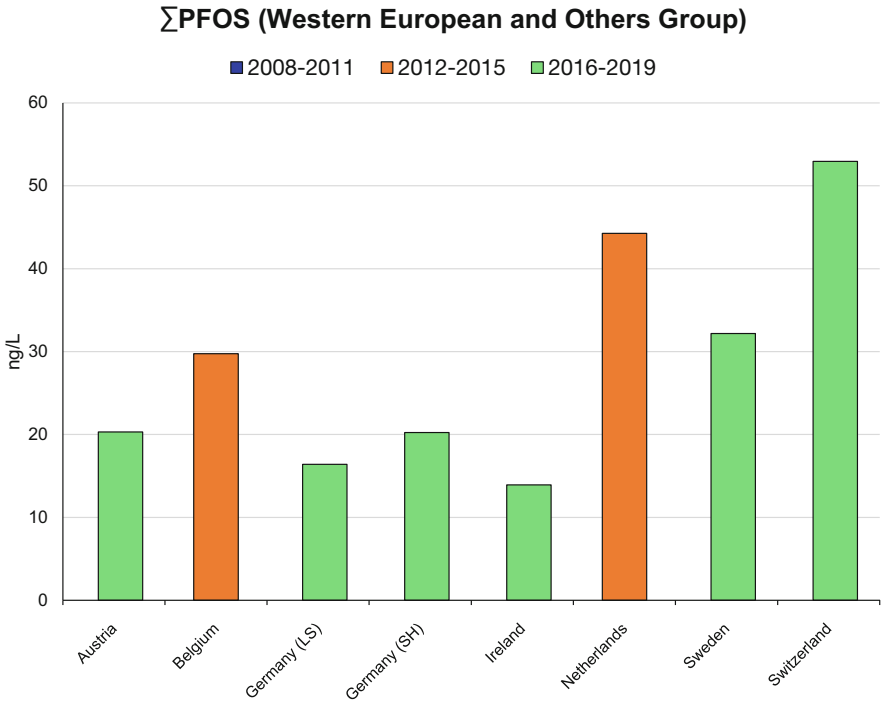

Fig. 17 Overview of the development of ∑PFOS concentrations in human milk (ng ∑PFOS/L) from Western European countries over time (period 2008–2011 in blue [however, no samples in this period], period 2012–2015 in orange, and period 2016–2019 in green)

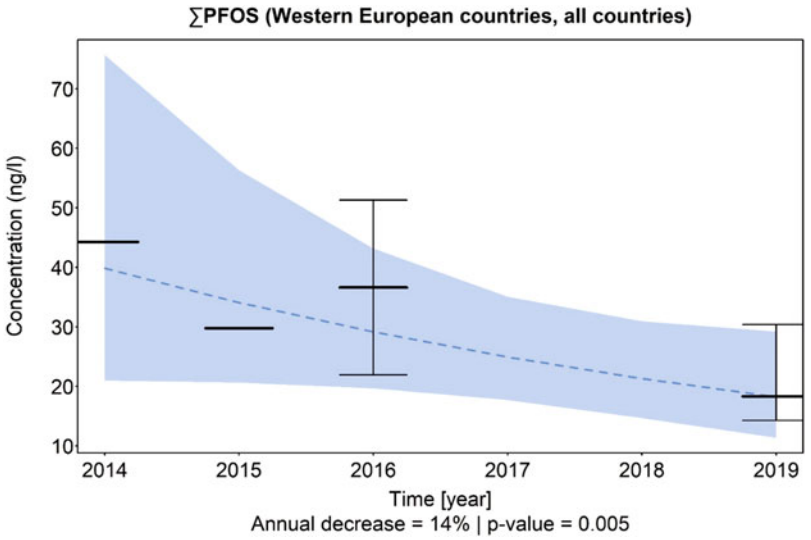

Fig. 18 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) in Western European countries (8 national pools, 7 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

Table 12 Overall decrease (%) of PFOS concentrations in Western European countries (computed using all samples)

Western Europe and Others Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All samples	7	14.5	–	79.1	–	0.005

3.1.7 Worldwide

The time trends of ∑PFOS concentrations derived by the Theil–Sen method from all 86 national pools from 59 countries are illustrated by Fig. 19 and for the 48 national pools from 24 countries with multiple participation by Fig. 20. The results from both approaches were comparable: Statistically significant decreases over 10 years for PFOS concentrations of 48.3% and 52.2%, respectively, were computed (1) using all samples and (2) using samples from countries with repeated participation (Table 13).

As described in subsection 3.1.1 above, the summarizing descriptive parameters seem to indicate an increase of the PFOS concentrations from 2008–2011 to 2012–2015 with a subsequent decrease to 2016–2019. The impact of countries

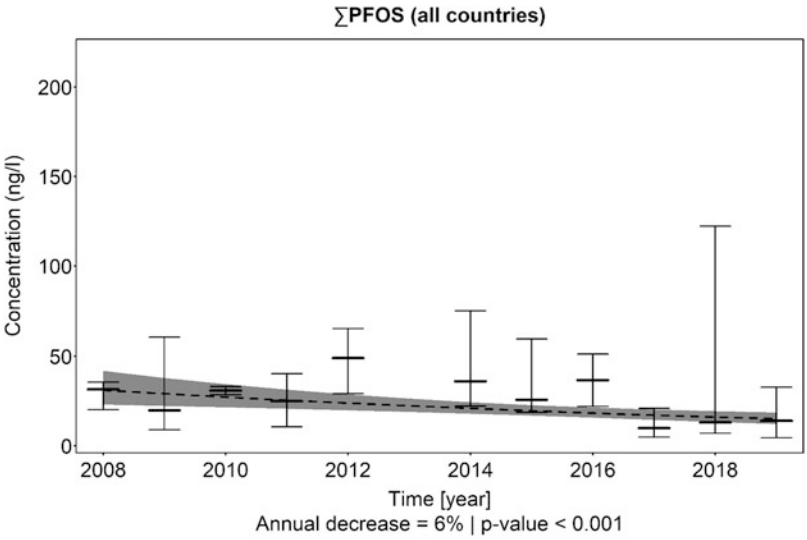

Fig. 19 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) using data from all countries (86 national pools, 59 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

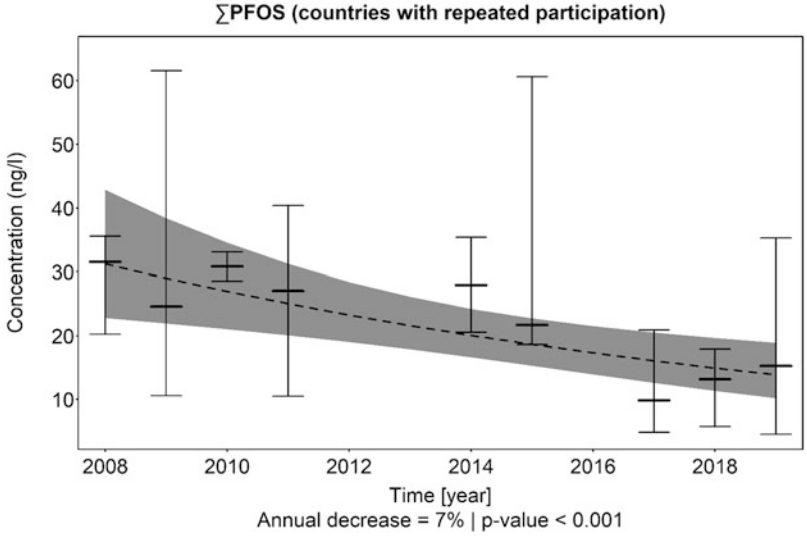

Fig. 20 Theil–Sen exponential trends of ∑PFOS concentrations in human milk (ng ∑PFOS/L) using data from countries with repeated participation (48 national pools, 24 countries)

Table 13 Overall decrease (%) of ∑PFOS concentrations worldwide computed (1) using all samples and (2) using samples from countries with repeated participation

Worldwide	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	59	6.4	9.4	48.3	62.6	<0.001
Repeatedly	24	7.1	7.2	52.2	52.6	<0.001

Table 14 Comparison of median, minimum, and maximum ∑PFOS concentrations (ng/L) in samples from countries participating in the 2012–2015 period: (1) countries with participation also in the 2008–2011 or 2016–2019 period; (2) countries participating only in the 2012–2015 period

	Countries with multiple participation	Countries with single participation only in 2012–2015 period
N	7	8
Median	21.7	40.1
Min	18.5	26.9
Max	64.3	85.8

participating in the 2012–2015 period on the obvious increase after listing in 2009 is assessed in the following in more details.

The lowest number of samples was available for this period (in total: 15 national pools). Seven samples came from countries with repeated participation (Côte d'Ivoire, Czech Republic, Georgia, Haiti, Lithuania, Moldova, Niger) and eight samples from countries with single participation (three samples from Brazil; Belgium, Bulgaria, Croatia, Netherlands, Romania). The range found in this period in all 15 samples (median 36.0 ng/L; range 18.5–85.8 ng/L) was clearly influenced by countries with single participation only in the 2012–2015 period: Minimum, median, and maximum concentrations were higher than in countries with multiple participation (Table 14).

The country-specific temporal trends for the seven countries with repeated participation including the 2012–2015 period as one of two periods between 2008 and 2019 were discussed above in the corresponding UN Regional Groups. Five of these countries had decreasing tendencies, one increasing and one quite constant concentration (Figs. 21 and 22). A decrease of 51% over 10 years was calculated for these seven countries, with a range between 6% and 82% for decrease rates over 10 years in Czechia, Georgia, Haiti, Lithuania, and Niger, quite constant concentrations in Moldova and an increase of 90% over 10 years in Côte d'Ivoire. As a result, the participation of different countries in the three rounds and the elevated contribution of samples from countries participating only in the 2012–2015 round explains the

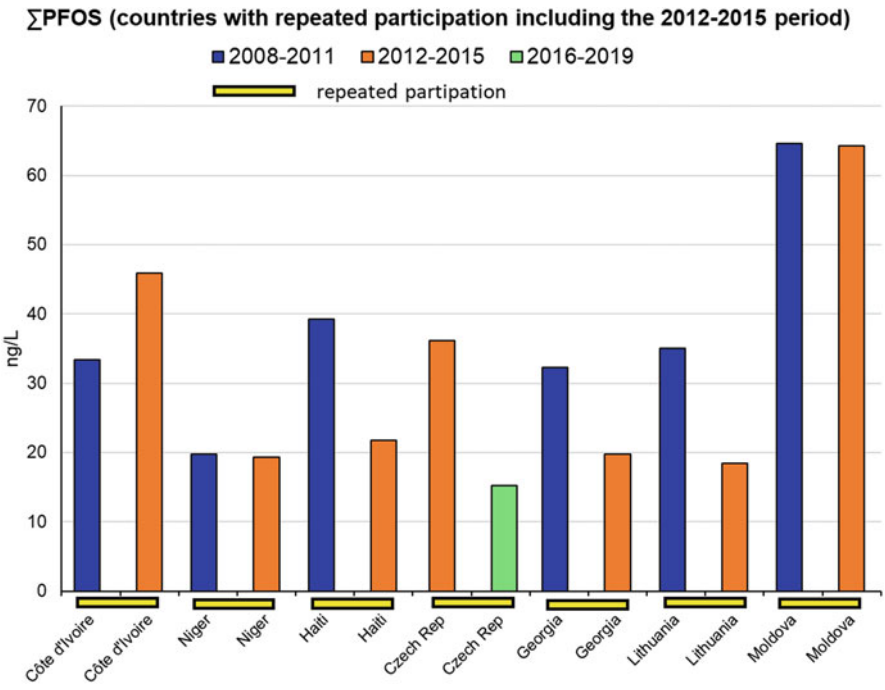

Fig. 21 Overview of the development of ∑PFOS concentrations in human milk (ng ∑PFOS/L) from seven countries with repeated participation including the 2012–2015 period as one of two periods between 2008 and 2019 (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods are marked by yellow rectangles)

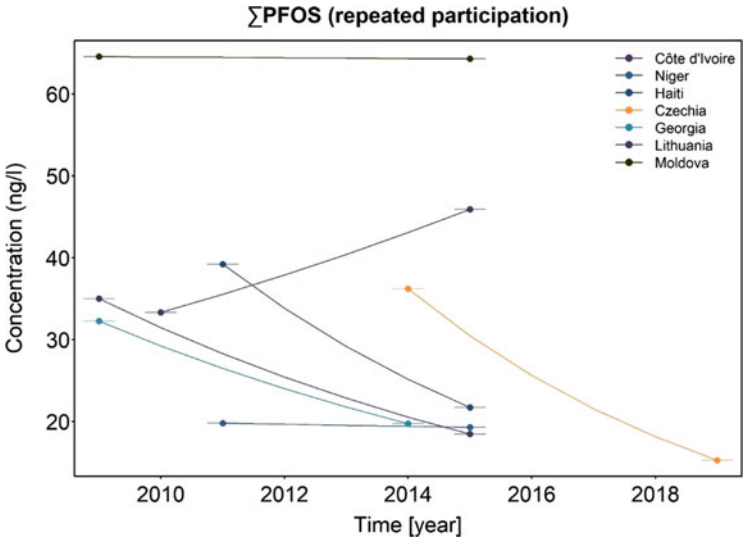

Fig. 22 Temporal tendencies of ∑PFOS concentrations (ng ∑PFOS/L) for seven countries with repeated participation including the 2012–2015 period as one of two periods between 2008 and 2019 using the Theil–Sen method

fluctuation of the descriptive parameters (e.g. median) between the three periods with an obvious maximum in the 2012–2015 period.

3.2 PFOA

PFOA was listed in Annex A of the Convention in 2019 (UNEP 2019b).

3.2.1 Estimation of Temporal Trends by Differentiation into Three Periods between 2008 and 2019

The median of the PFOA concentrations of 86 national pools from 59 countries increased from 21.5 ng/L in the 2008–2011 period ($N = 27$) to 32.1 ng/L in the 2012–2015 period ($N = 15$) and then decreased to 16.3 ng/L in the 2016–2019 period ($N = 44$). The highest maximum level (65.3 ng/L) was found in the 2008–2011 period (Fig. 23). However, as described above for PFOS, it has to be checked also for PFOA whether these fluctuations of the median are likely due to the result of participation of different countries in different rounds of the WHO/UNEP-coordinated exposure studies.

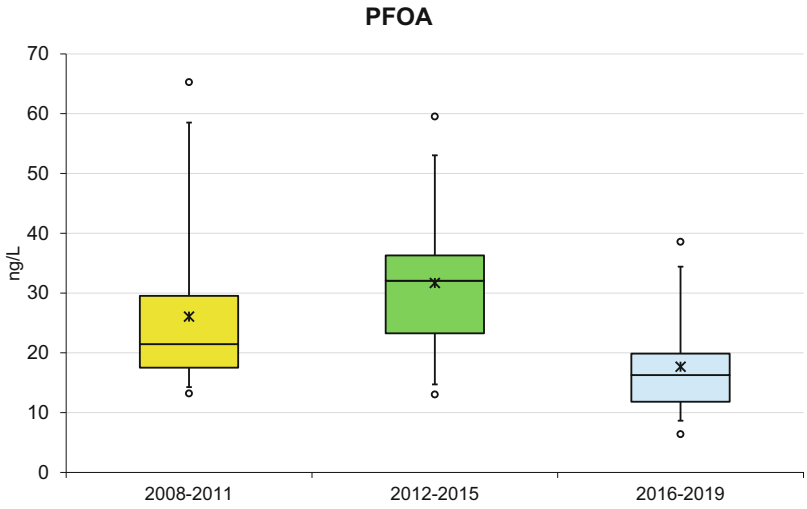

Fig. 23 Range of PFOA concentrations (ng/L) in three periods ($N = 27$ in 2008–2011; $N = 15$ in 2012–2015; $N = 44$ in 2016–2019) [box plot; minimum and maximum: as circles; fifth and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

3.2.2 African Group

In the 16 African countries, PFOA concentrations between 2008 and 2019 were in a range 6.4–65.3 ng/L. In two of the 11 countries with repeated participation, PFOA concentrations remained quite constant (Côte d'Ivoire; Nigeria). In 9 countries, downward tendencies were observed, with a decrease by 31% as median of the observed decreases between the first and second submission (without normalization, e.g. to a 10-year period) and the highest decrease by 87% (Uganda, from 2008 to 2018) (Fig. 24).

Overall, levels decreased from 18.0 ng/L as median (range 14.0–65.3 ng/L; $N = 11$) in 2008–2011 to 12.8 ng/L as median (range 6.4–18.6 ng/L; $N = 14$) in 2016–2019.

Figure 25 illustrates the temporal tendencies in 11 countries with multiple participation using the Theil–Sen method (comprising all individual pooled samples and assuming exponential trends, see subsection 2.2). Overall decreases per 1 year and 10 years are given in Table 15. The limited number of samples did not allow to determine statistically significant decreases for countries ($p \sim 1.000$). As median of these countries, the levels of PFOA in African countries decreased within a 10-year period by 37%. This is in line with the statistically significant ($p < 0.001$) decrease over 10 years of 32.5% for this UN region derived by countries with multiple

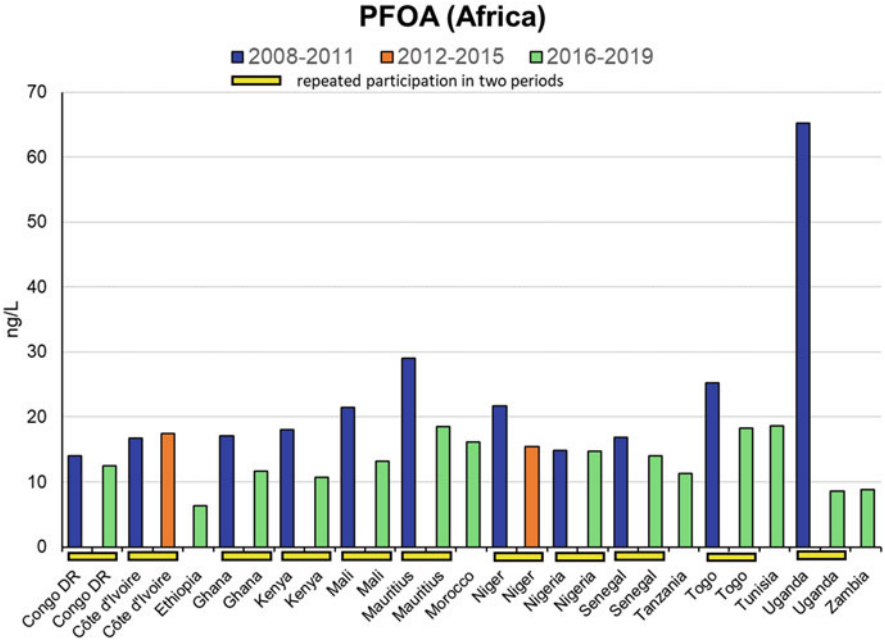

Fig. 24 Overview of the development of PFOA concentrations in human milk (ng/L) from African countries over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles)

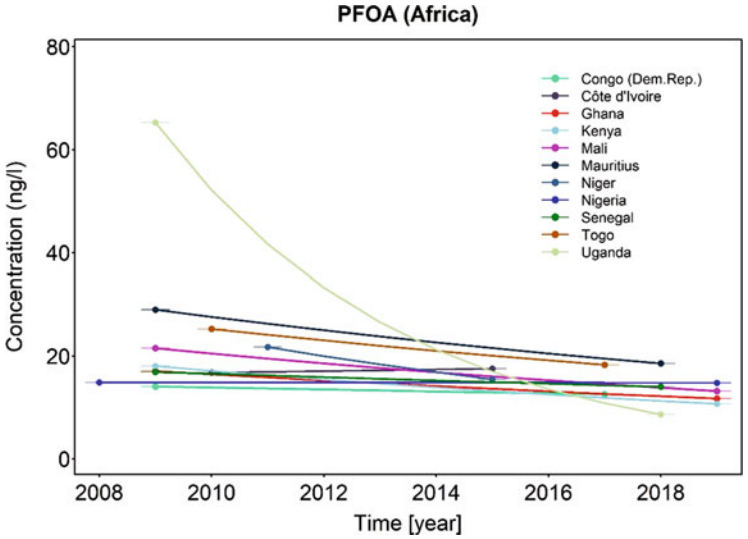

Fig. 25 Temporal tendencies of PFOA concentrations (ng/L) for African countries with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 15 Overall decrease (%) of PFOA concentrations per 1 year and 10 years in African countries (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Congo (DR)	1.4	13.4	1.000
Côte d'Ivoire	−0.9	−9.4	1.000
Ghana	3.7	31.5	1.000
Kenya	5.1	40.8	1.000
Mali	4.8	38.7	1.000
Mauritius	4.9	39.2	1.000
Niger	8.1	57.3	1.000
Nigeria	0.0	0.4	1.000
Senegal	2.1	18.8	1.000
Togo	4.5	37.0	1.000
Uganda	20.1	89.5	1.000
Median	4.5	37.0	

participation and calculated by the Theil–Sen method (see Table 16 in the following).

Statistically significant time trends can be derived in the African Regional Group by pooling of data from countries. The time trends of PFOA concentrations derived by the Theil–Sen method are illustrated by Fig. 26 for all 27 national pools from

Table 16 Overall decrease (%) of PFOA concentrations in the African Group computed (1) using all samples and (2) using samples from countries with repeated participation

African Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	16	4.5	4.5	36.9	37.0	<0.001
Repeatedly	11	3.9	4.5	32.5	37.0	<0.001

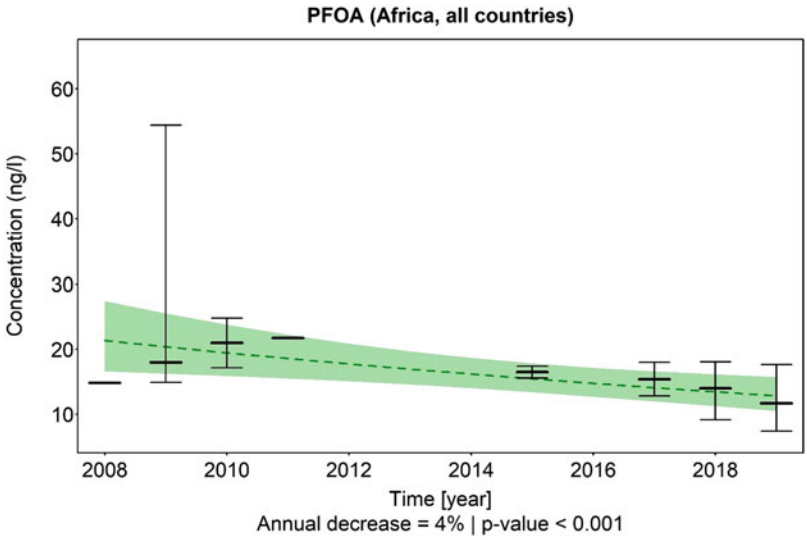

Fig. 26 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) in all African countries (27 national pools, 16 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

16 countries and by Fig. 27 for 22 national pools from 11 countries with multiple participation. Overall decreases over 10 years for PFOA concentrations in the African Group of 37% and 33%, respectively, were achieved computed (i) using all samples and (ii) using samples from countries with repeated participation (Table 16).

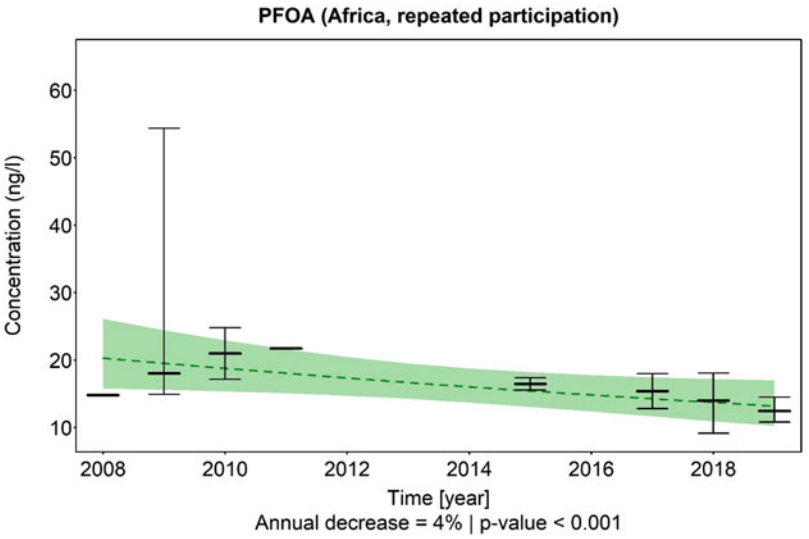

Fig. 27 Theil–Sen exponential trends of PFOA in human milk (ng/L) in African countries with repeated participation (22 national pools, 11 countries)

3.2.3 Asia-Pacific Group

From the 16 countries of the Asia-Pacific region, data covering two periods were available only for Fiji showing a reduction of 70% from 2011 (36 ng/L) to 2019 (10.5 ng/L). In the most recent period (2016–2019), all countries had PFOA concentrations in a range between 10 and 33 ng/L (median 15.1 ng/L; $N = 13$), in comparison to a range between 13 and 36 ng/L (median 20.6 ng/L; $N = 5$) in the 2008–2011 period (Fig. 28).

Figure 29 illustrates the temporal tendencies in the two countries with multiple participation using the Theil–Sen method. The limited number of samples did not allow to determine a statistically significant decrease for Fiji ($p \sim 1.000$) (Table 17). The short period between the two samplings in Niue (2017 and 2019) should not be used for calculation of temporal trends over a 10 year-period.

Statistically significant time trends of PFOA concentrations in the Asia-Pacific region were derived by the Theil–Sen method combining all 18 national pools from 16 countries (Fig. 30). As explained above, regional trends cannot be derived based on countries with multiple participation due to lack of a sufficient number of countries. An overall decrease over 10 years of 38.6% was computed by the Theil–Sen method using all samples (Table 18).

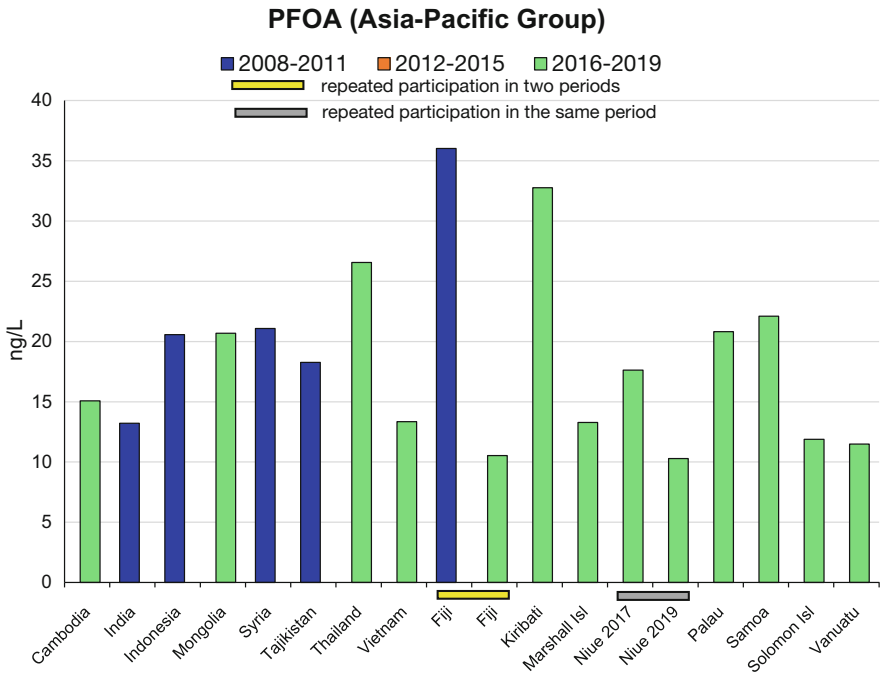

Fig. 28 Overview of the development of PFOA concentrations in human milk (ng PFOA/L) from countries of the Asia-Pacific Group over time (period 2008–2011 in blue, period 2012–2015 in orange [however, no samples in this period], and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles, with repeated participation in the same period by grey rectangles)

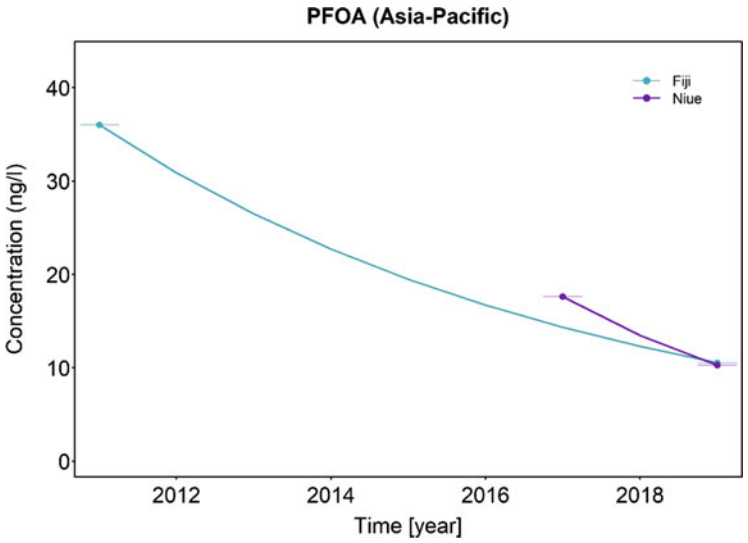

Fig. 29 Temporal tendencies of PFOA concentrations (ng/L) for countries of the Asia-Pacific Group with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 17 Overall decrease (%) of PFOA concentrations per 1 year and 10 years in Fiji (calculated by the Theil–Sen method)

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Fiji	14.2	78.5	1.000

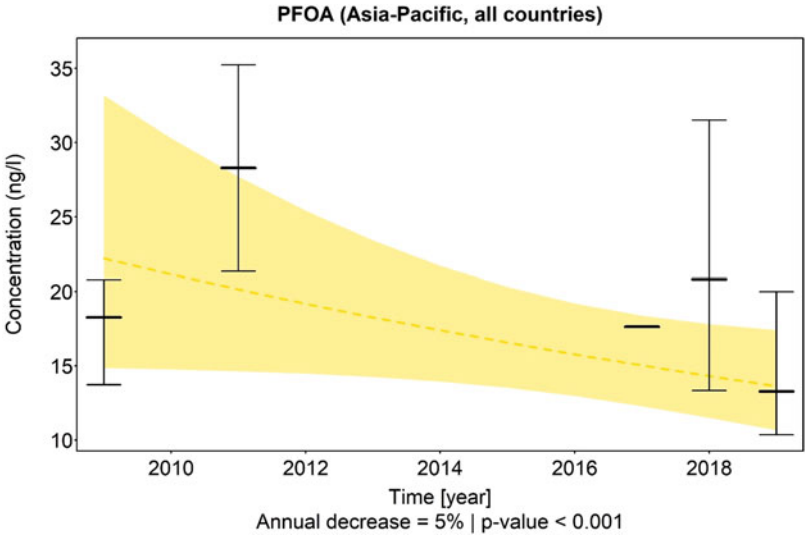

Fig. 30 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) in countries of the Asia-Pacific group (18 national pools, 16 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

Table 18 Overall decrease (%) of PFOA concentrations in the Asia-Pacific Group computed using all samples (n.a. = not applicable)

Asia-Pacific Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All samples	16	4.8	n.a.	38.6	n.a.	<0.001

3.2.4 Group of Latin American and Caribbean Countries (GRULAC)

In the 12 GRULAC countries, PFOA concentrations between 2008 and 2019 were in the range 8.0–63.3 ng/L, with downward tendencies in 5 countries from the 2008–2011 period to the 2016–2019 period. In these 5 countries (Antigua-Barbuda, Jamaica, Mexico, Peru, and Uruguay), PFOA concentrations decreased by 47% as median of the observed decrease rates between the first and second submission (without normalization, e.g. to a 10-year period) from 2008–2011 to 2016–2019. The considerable differences in PFOA concentrations in the two samples from Chile (2008; 2011) collected in the same period are presumably caused by differences in the regional origin of these two samples, as concluded from discussion of WHO-TEQ and PCDD/PCDF patterns samples (Malisch et al. 2023d) and not the result of decreasing trends over a period of three years. In Haiti, an upward tendency was observed from 2011 to 2015 (Fig. 31).

Overall, levels decreased from 24.5 ng/L as median (range 15.6–63.3 ng/L; $N = 8$) in 2008–2011 to 16.4 ng/L as median (range 8.0–19.6 ng/L; $N = 9$) in 2016–2019, with a higher median of 29.9 ng/L in the 2012–2015 period (range 26.1 to 35.2 ng/L; $N = 4$). As explained above already for PFOS in this UN region, the inclusion of different countries in different periods limits the comparability of

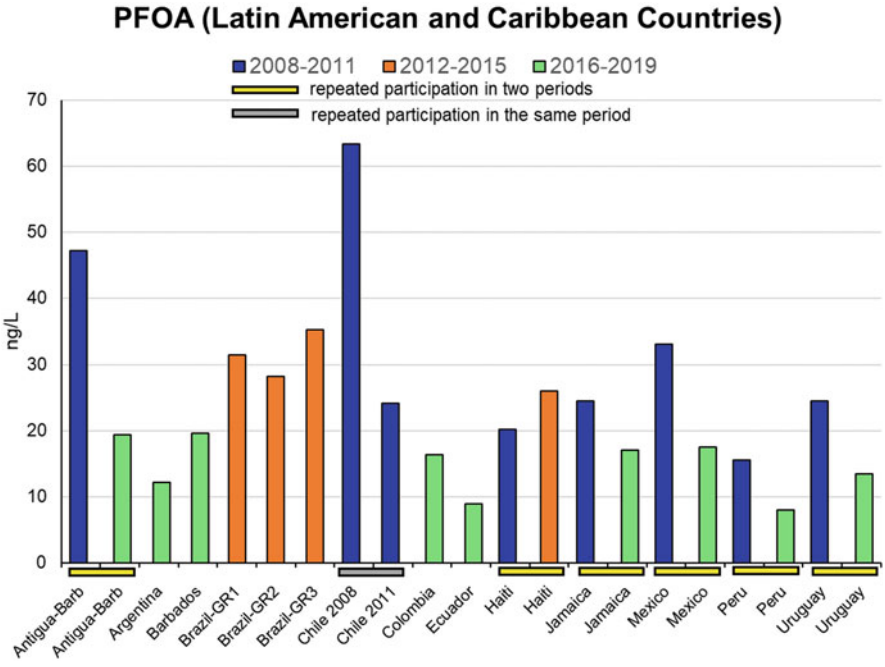

Fig. 31 Overview of the development of PFOA concentrations in human milk (ng/L) from Latin American and Caribbean countries over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles, with repeated participation in the same period by grey rectangles)

results. The four data for the 2012–2015 period were essentially influenced by the three results from one country (Brazil). However, temporal trends cannot be derived for Brazil, as only results for three samples of 2012 are available.

Figure 32 illustrates the temporal tendencies of PFOA in human milk from the seven countries with multiple participation using the Theil–Sen method. Overall decrease rates per 1 year and 10 years are given in Table 19. The limited number of samples did not allow to determine statistically significant decreases for these Latin American and Caribbean countries ($p \sim 1.000$). As median, the levels of PFOA in these countries decreased within a 10-year period by 51%. This is in line with the statistically significant ($p < 0.001$) decrease over 10 years of 65% for this UN region

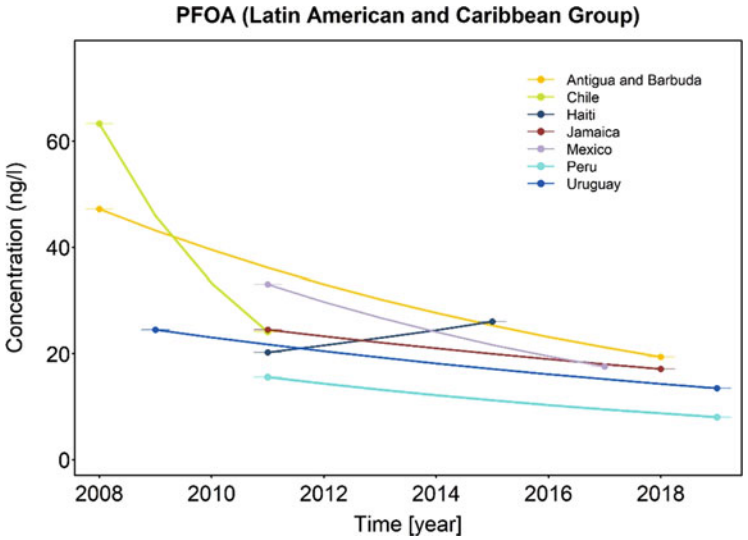

Fig. 32 Temporal tendencies of PFOA concentrations (ng/L) for Latin American and Caribbean countries with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 19 Overall decrease (%) of PFOA concentrations per 1 year and 10 years in Latin American and Caribbean countries (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Antigua-Barb.	8.5	58.9	1.000
Haiti	−6.5	−87.9	1.000
Jamaica	5.0	40.2	1.000
Mexico	10.0	65.2	1.000
Peru	7.9	56.2	1.000
Uruguay	5.8	45.1	1.000
Median	7.9	50.6	

derived by countries with multiple participation and calculated by the Theil–Sen method (see Table 20 in the following).

Time trends of PFOA concentrations in this region derived by the Theil–Sen method are illustrated by Fig. 33 for all 21 national pools from 12 countries and by Fig. 34 for 14 national pools from 7 countries with multiple participation. Statistically significant decreases over 10 years of 63% and 65%, respectively, were

Table 20 Overall decrease (%) of PFOA concentrations in Latin American and Caribbean countries computed (1) using all samples and (2) using samples from countries with repeated participation

Latin America and Caribbean	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	12	9.5	7.9	63.0	50.6	<0.001
Repeatedly	7	9.8	7.9	64.5	50.6	<0.001

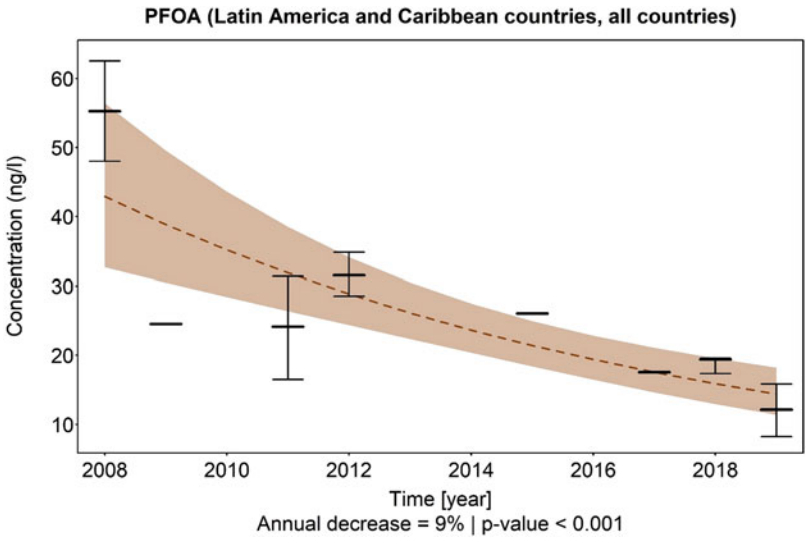

Fig. 33 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) in Latin American and Caribbean countries (21 national pools, 12 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

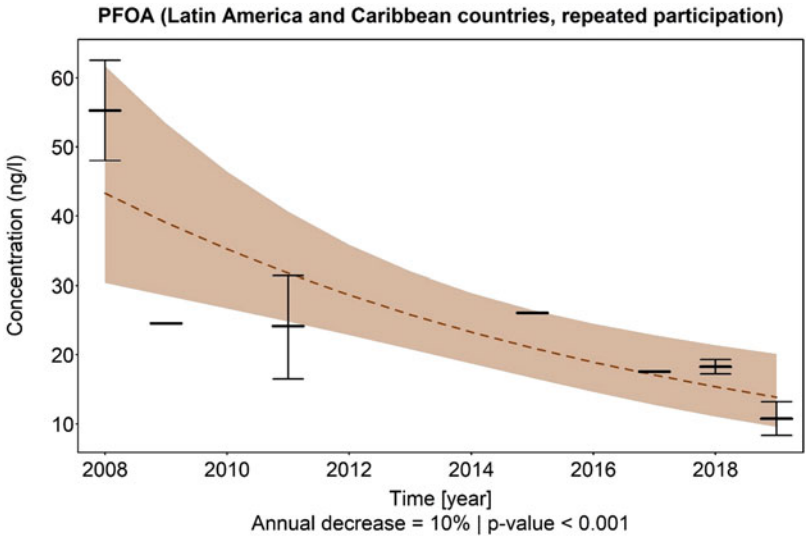

Fig. 34 Theil–Sen exponential trends of PFOA in human milk (ng/L) in Latin American and Caribbean countries with repeated participation (14 national pools, 7 countries)

achieved computed (1) using all samples and (2) using samples from countries with repeated participation (Table 20).

3.2.5 Eastern European Group

In 12 national pools from 8 Eastern European countries, PFOA concentrations between 2008 and 2019 were in the range 13.0–50.3 ng/L, with increasing concentrations from 2009 until 2015 by 12% in Lithuania, and by 67% in Moldova, with downward tendencies from 2009 until 2014 by 37% in Georgia, and with downward tendencies from 2014 to 2019 by 37% in the Czech Republic (Fig. 35). Overall, the summarizing parameters seem to indicate quite constant PFOA levels with 30.1 ng/L as median ($N = 3$; range 20.6–31.2 ng/L) in 2008–2011, 34.9 ng/L as median ($N = 7$; range 13.0–50.3 ng/L) in 2012–2015, and 27.5 ng/L as median ($N = 2$; range 25.4–29.5 ng/L) in 2016–2019.

Figure 36 illustrates the temporal tendencies in the four countries with multiple participation using the Theil–Sen method. Overall decreases per 1 year and 10 years are given in Table 21. The limited number of samples did not allow to determine statistically significant decreases for countries ($p \sim 1.000$). Whereas in the Czech Republic and Georgia an overall decrease over 10 years of 60% was found, a slight increase was observed in Lithuania and a clear increase in Moldova.

The inconsistent temporal tendencies of these four countries with multiple participation are reflected by the lack of statistical significance after pooling of data from

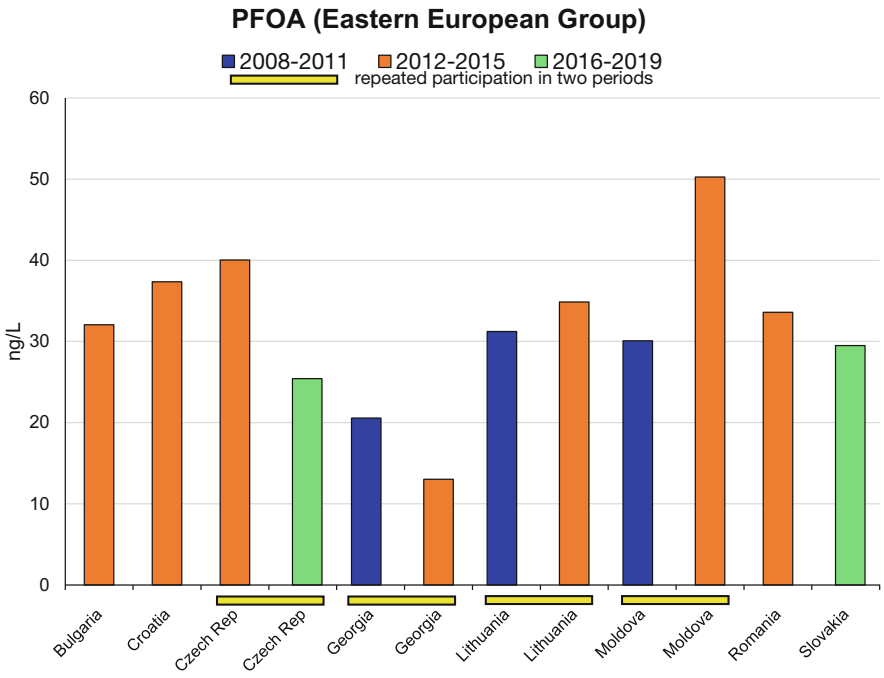

Fig. 35 Overview of the development of PFOA concentrations in human milk (ng/L) from countries of the Eastern European Group over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles)

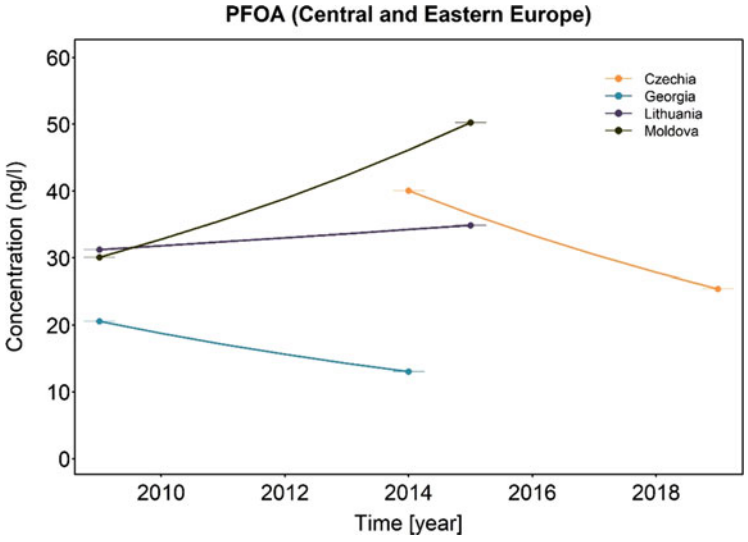

Fig. 36 Temporal tendencies of PFOA concentrations (ng/L) for countries of the Eastern European Group with repeated participation between 2008 and 2019 using the Theil–Sen method

Table 21 Overall decrease (%) of PFOA concentrations per 1 year and 10 years in human milk from countries of the Eastern European Group (calculated by the Theil–Sen method). Negative decreases are to be read as increase

Country	Overall decrease (%) per 1 year	Overall decrease (%) per 10 years	Trend *p*-value overall
Czechia	8.7	59.7	1.000
Georgia	8.7	59.9	1.000
Lithuania	−1.9	−20.2	1.000
Moldova	−8.9	−135.3	1.000
Median	3.4	19.8	

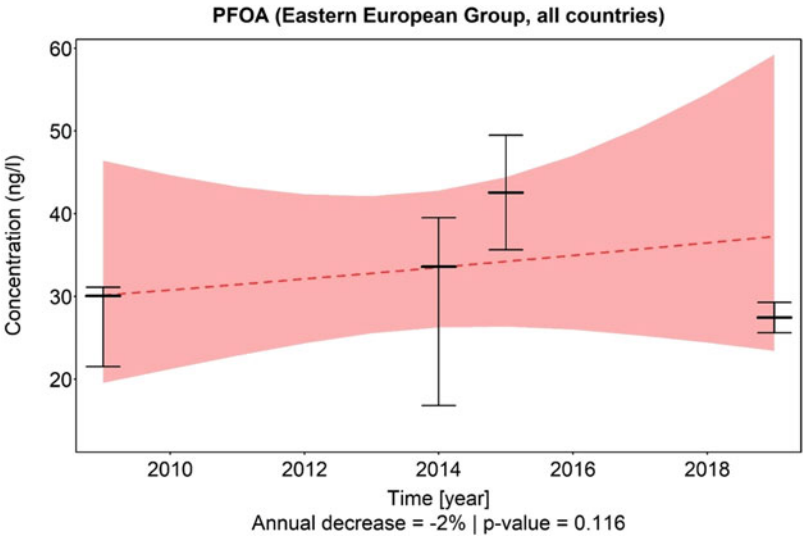

Fig. 37 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) from countries of the Eastern European Group (12 national pools, 8 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

these countries and use of the Theil–Sen method (Fig. 37 for all 12 national pools from 8 countries; Fig. 38 for the 8 national pools from 4 countries with multiple participation). Furthermore, discrepancies between the Theil–Sen method and the median method indicate difficulties in assessing statistically significant temporal trends for overall temporal tendencies over 10 years for PFOA (Table 22).

Using the Hites method of break point search (Hites 2019) on all countries in the Eastern European Group, a break point was found for PFOA, although, the result was non-significant.

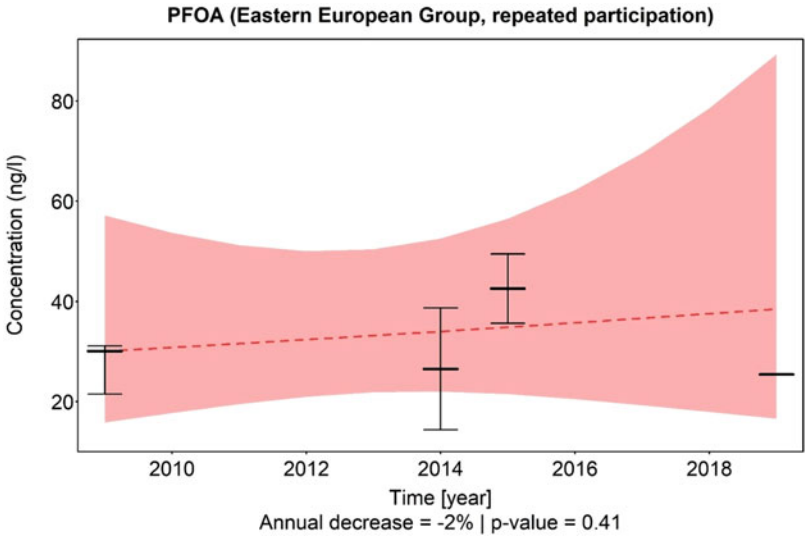

Fig. 38 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) in countries of the Eastern European Group with repeated participation (8 national pools, 4 countries)

Table 22 Overall decrease (%) of PFOA concentrations in the Eastern European Group (EEG) computed (1) using all samples and (2) using samples from countries with repeated participation. Negative decreases are to be read as increase

Eastern European Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	8	−2.1	3.6	−23.6	19.8	0.116
Repeatedly	4	−2.5	3.6	−27.9	19.8	0.41

3.2.6 Western European and Others Group (WEOG)

In eight national pools from seven Western European countries, PFOA concentrations between 2014 and 2019 were in a range 18.2–59.5 ng/L. There were no countries with multiple participation (Fig. 39). Time trends derived by the Theil–Sen method are illustrated by Fig. 40. No statistically significant time trend can be derived in this UN regional group by pooling of all data from countries. An overall decrease over 10 years of 58% was computed using all samples (Table 23).

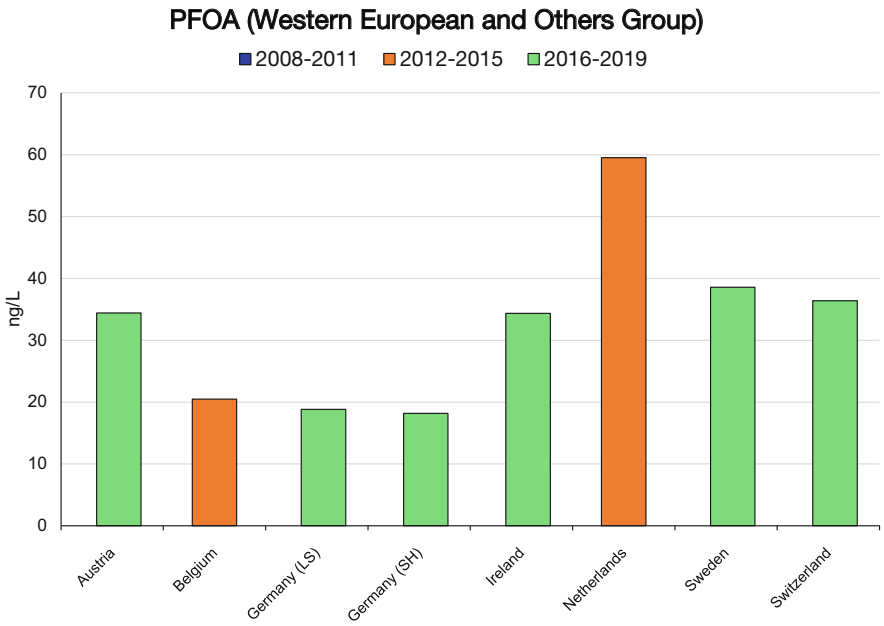

Fig. 39 Overview of the development of PFOA concentrations in human milk (ng/L) from countries of the Western European and Others Group over time (period 2008–2011 in blue [without samples in this period], period 2012–2015 in orange, and period 2016–2019 in green)

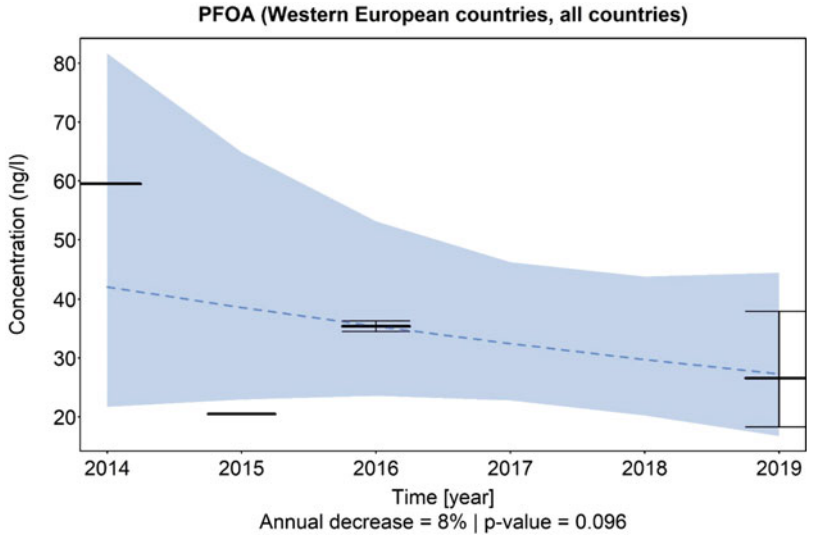

Fig. 40 Theil–Sen exponential trends of PFOA in human milk (ng/L) in countries of the Western European Group and Others Group (8 national pools, 7 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

Table 23 Overall decrease (%) of PFOA concentrations in Western European countries (computed using all samples)

Western Europe and Others Group	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All samples	7	8.3	–	58	–	0.096

3.2.7 Worldwide

The time trends of PFOA concentrations derived by the Theil–Sen method from all 86 national pools from 59 countries are illustrated by Fig. 41 and for the 48 national pools from 24 countries with multiple participation by Fig. 42. The results from both approaches were comparable: Statistically significant decreases over 10 years for PFOA concentrations of 41.7% and 46.6%, respectively, were calculated by the Theil–Sen method (1) using all samples and (2) using samples from countries with repeated participation (Table 24).

The summarizing descriptive parameters of Fig. 23 in subsection 3.2.1 seem to indicate an increase of PFOA concentrations from 2008–2011 to 2012–2015 with a subsequent decrease to 2016–2019. The lowest number of samples was available for

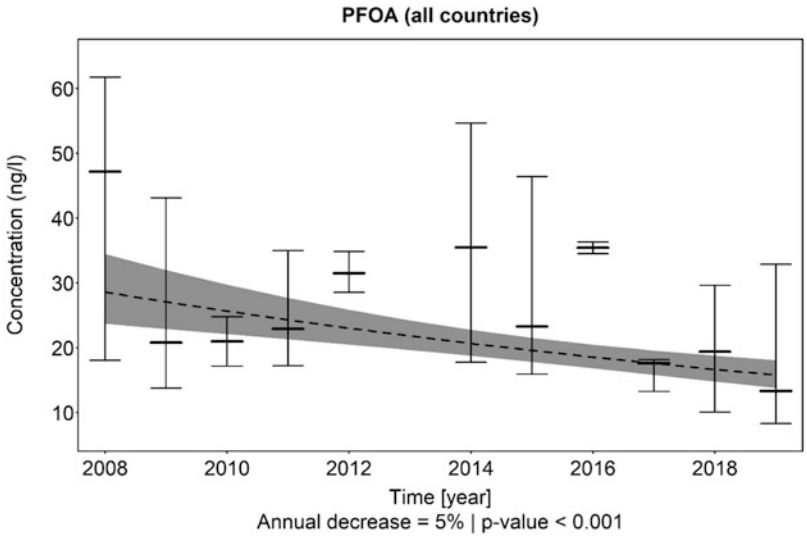

Fig. 41 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) worldwide using data from all countries (86 national pools, 59 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles.)

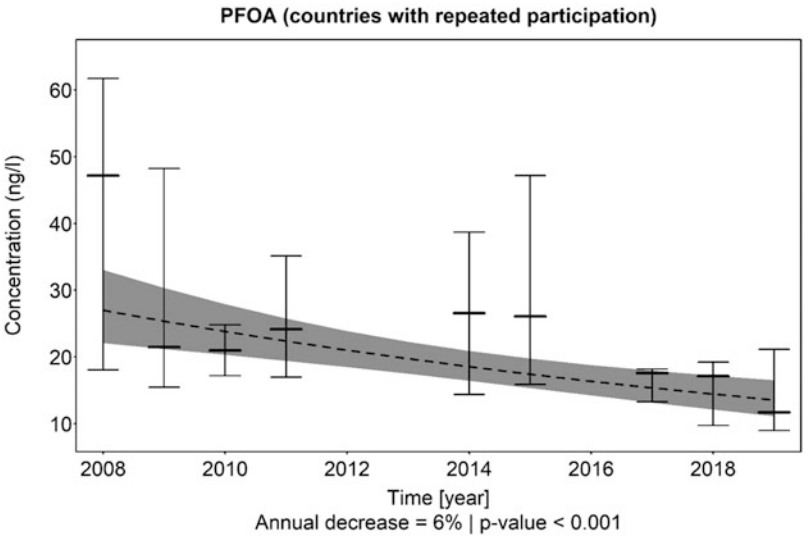

Fig. 42 Theil–Sen exponential trends of PFOA concentrations in human milk (ng/L) worldwide using data from countries with repeated participation (48 national pools, 24 countries)

Table 24 Overall decrease (%) of PFOA concentrations worldwide computed (1) using all samples and (2) using samples from countries with repeated participation

Worldwide	*N* of countries	Overall decrease (%) per 1 year		Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	Theil–Sen method	Median method	
All countries	59	5.3	4.8	41.7	38.6	<0.001
Repeatedly	24	6.1	6.9	46.6	51.0	<0.001

the 2012–2015 period (in total: 15 national pools). 7 samples came from countries with repeated participation (Côte d'Ivoire, Czech Republic, Georgia, Haiti, Lithuania, Moldova, Niger) and 8 samples from countries with single participation (three samples from Brazil; Belgium, Bulgaria, Croatia, Netherlands, Romania). The range found in this period in all 15 samples (median 36.0 ng/L; range 18.5–85.8 ng/L) was clearly influenced by countries with single participation only in the 2012–2015 period: Minimum, median, and maximum concentrations in the 2012–2015 period were higher in countries participating only in this period than in countries with multiple participation (Table 25). Therefore, the participation of different countries in the three rounds and the elevated contribution of samples

Table 25 Comparison of median, minimum, and maximum PFOA concentrations (ng/L) in the 2012–2015 period of (1) countries with participation also in the 2008–2011 or 2016–2019 period; (2) countries participating only in the 2012–2015 period

	Countries with multiple participation	Countries with single participation only in 2012–2015 period
N	7	8
Median	26.1	32.8
Min	13.0	20.5
Max	50.3	59.5

from countries participating only in the 2012–2015 round contributes considerably to the fluctuation of the descriptive parameters (e.g. median) between the three periods with an obvious maximum in the 2012–2015 period.

The country-specific temporal trends for the seven countries with repeated participation including the 2012–2015 period as one of two periods between 2008 and 2019 were discussed above in the corresponding UN Regional Groups. Inconsistent temporal tendencies of these seven countries were found: Three of these countries had clearly decreasing tendencies (decrease rates over 10 years between 57% and 60% in Niger, Czechia, and Georgia), two a slightly increasing tendency (Côte d'Ivoire, −9.4%; Lithuania, −20.2%), and two clearly increasing tendencies (Haiti, −87%; Moldova, −135%) (for illustration, see Figs. 43 and 44).

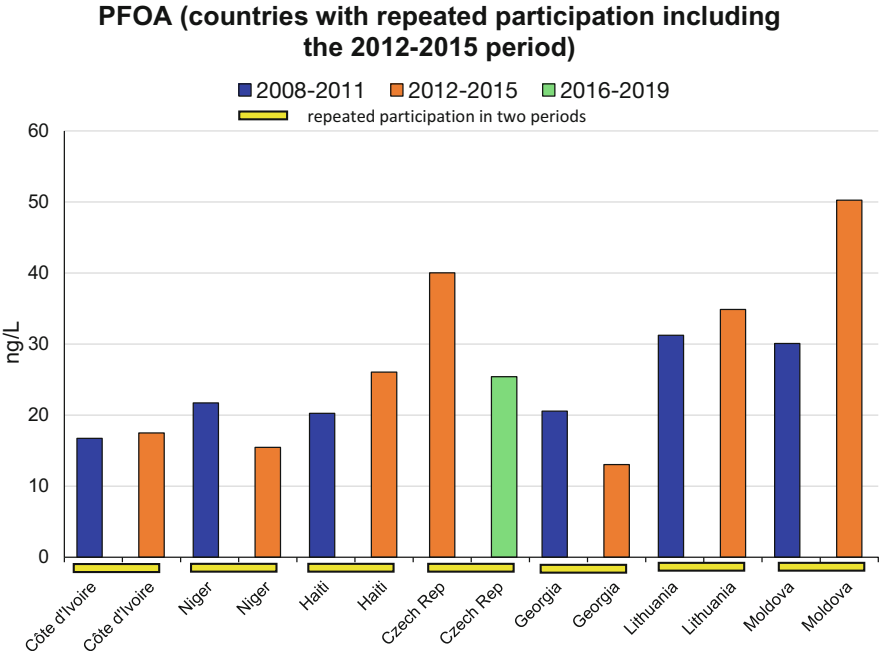

Fig. 43 Overview of the development of PFOA concentrations in human milk (ng/L) for countries with repeated participation including the 2012–2015 period as one of two periods between 2008 and 2019

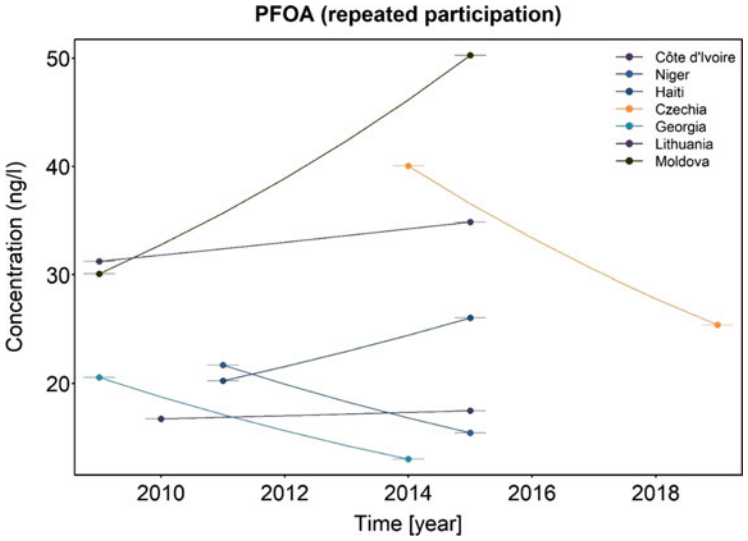

Fig. 44 Temporal tendencies of PFOA concentrations (ng/L) for countries with repeated participation including the 2012–2015 period as one of two periods between 2008 and 2019 using the Theil–Sen method

3.3 PFHxS

An initial indicative list of PFHxS, its salts and PFHxS-related compounds was presented to the Convention of Parties in 2019 (UNEP 2019c). PFHxS was then listed in Annex A of the Convention in 2022 (UNEP 2022).

In 84% of the 86 samples from 59 countries, PFHxS concentrations were below the limit of quantification (LOQ; 5.7 ng/L). 11 countries had PFHxS concentrations (slightly) above the LOQ (range 5.7–10 ng/l). Four samples had concentrations above 10 ng/L with one country more than 10-times higher levels (13.3 ng/L [Romania, 2014]; 17.9 ng/L [Sweden, 2019]; 35.8 ng/L [Haiti, 2011]; 115 ng/L [Kiribati, 2018]). The high rate of samples with concentrations below LOQ impedes the assessment of time trends.

3.3.1 African Group

From 27 samples of 16 countries, 25 samples were below LOQ (< 5.7 ng/L); one country had a concentration in the range of LOQ (Togo, 2010: 5.8 ng/L), one country above LOQ (Mali 2009: 7.8 ng/L) (Fig. 52 in the appendix). In nearly all countries with repeated participations, the PFHxS concentrations were below LOQ for both participations. Therefore, no temporal tendencies can be derived, neither in general between periods based on results of all countries, nor by combining data from all countries and use of the Theil–Sen method (Fig. 45) and country-specific for countries with repeated participation except for Mali (decrease from 2009 [7.8 ng/L]

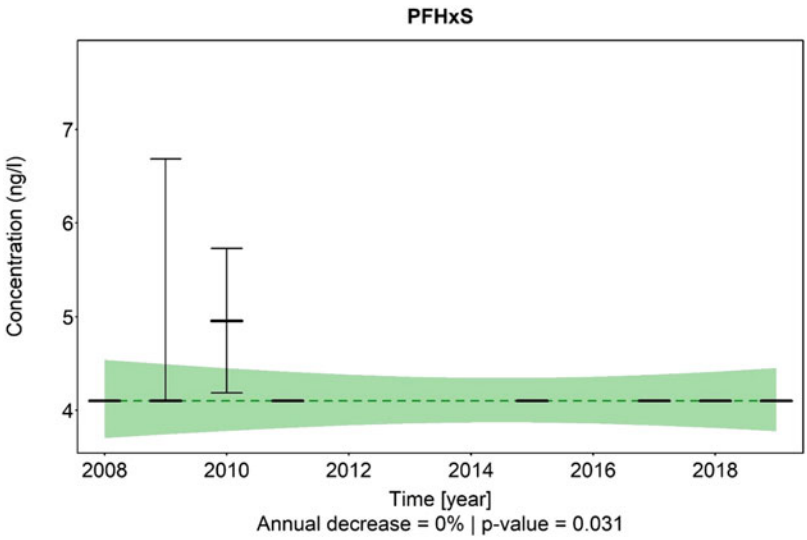

Fig. 45 Theil–Sen exponential trends of PFHxS concentrations in human milk (ng/L) from all African countries (27 national pools, 16 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles. To replace left-censored values, values under LOQ were substituted by 1/sqrt(2) of the LOQ.)

to 2019 [<LOQ]). The changes in Togo from 5.8 ng/L in 2010 to <5.7 in 2017 are in the range of the LOQ and cannot be used to estimate temporal trends quantitatively.

3.3.2 Asia-Pacific Group

From 18 samples of 16 countries, 15 samples were below LOQ (< 5.7 ng/L); one country had a concentration in the range of the LOQ (Fiji, 2011: 5.9 ng/L), one country of 7.5 ng/L (Thailand, 2018), and one country 115 ng/L (Kiribati, 2018) (Fig. 53 in the appendix). The changes in Fiji from 5.9 ng/L in 2011 to <5.7 in 2019 are in the range of the LOQ and cannot be used to estimate any temporal tendencies. Both samples from Niue (collected in 2017 and 2019) were below LOQ. Therefore, no temporal tendencies can be derived, neither in general between periods based on results of all countries, nor by combining data from all countries and use of the Theil–Sen method (Fig. 46) nor country-specific for countries with repeated participation.

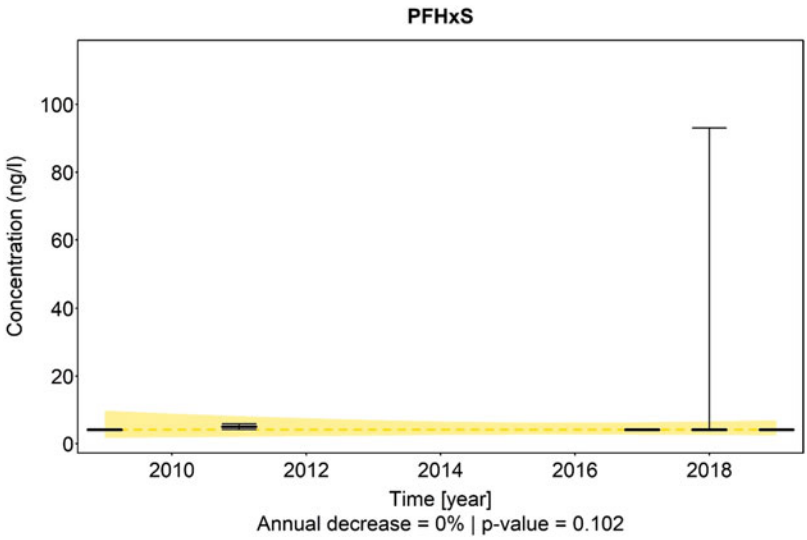

Fig. 46 Theil–Sen exponential trends of PFHxS concentrations in human milk (ng/L) in countries of the Asia-Pacific group (18 national pools, 16 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles. To replace left-censored values, values under LOQ were substituted by 1/sqrt(2) of the LOQ.)

3.3.3 Group of Latin American and Caribbean Countries (GRULAC)

From 21 samples of 12 countries, 17 samples were below LOQ (<5.7 ng/L). From six countries with repeated participation in two periods, in three countries the PFHxS concentrations were below LOQ in both periods. In two countries, PFHxS concentrations had decreasing tendencies from a concentration quite close to the LOQ to below LOQ (Antigua-Barbuda, 2008: 6.6 ng/L, 2018: <5.7 ng/L; Jamaica, 2011: 6.0 ng/L, 2018: <5.7 ng/L). In one country, a considerable decrease was found (Haiti, 2011: 35.8 ng/L, 2015: 8 ng/L) (Fig. 54 in the appendix). Therefore, temporal tendencies cannot be derived in general between periods based on results of all countries, nor by combining data from all countries and use of the Theil–Sen method (Fig. 47). On a country-specific basis, a considerable decrease was found in Haiti.

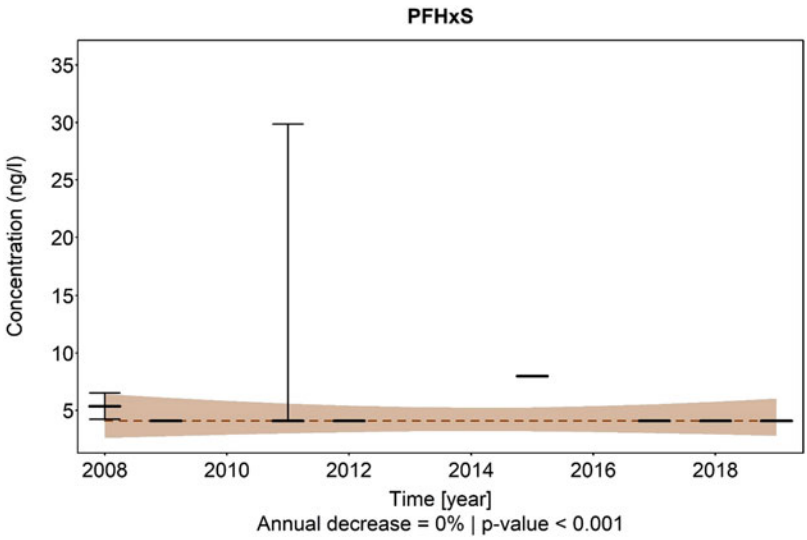

Fig. 47 Theil–Sen exponential trends of PFHxS concentrations in human milk (ng/L) in Latin American and Caribbean countries (21 national pools, 12 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles. To replace left-censored values, values under LOQ were substituted by 1/sqrt(2) of the LOQ.)

3.3.4 Eastern European Group

From 12 samples of 8 countries, 9 samples were below LOQ (<5.7 ng/L). One country had a PFHxS concentration in the range of the LOQ (Croatia, 2014: 6.1 ng/L) and two countries above (Moldova, 2015: 9.9 ng/L; Romania, 2014: 13.3 ng/L) (Fig. 55 in the appendix). Therefore, no general temporal tendencies can be derived by comparing periods, nor by combining data from all countries and use of the Theil–Sen method (Fig. 48). From four countries with repeated participation, an increasing tendency was observed in Moldova from 2009 to 2015 (from <LOQ to 9.9 ng/L), whereas in the other three countries (Czech Republic, Georgia, Lithuania) PFHxS concentrations below LOQ were found in both periods.

Using the Hites method of break point search (Hites 2019) on all countries in the Eastern European Group, a break point was found for PFHxS, although, the result was non-significant.

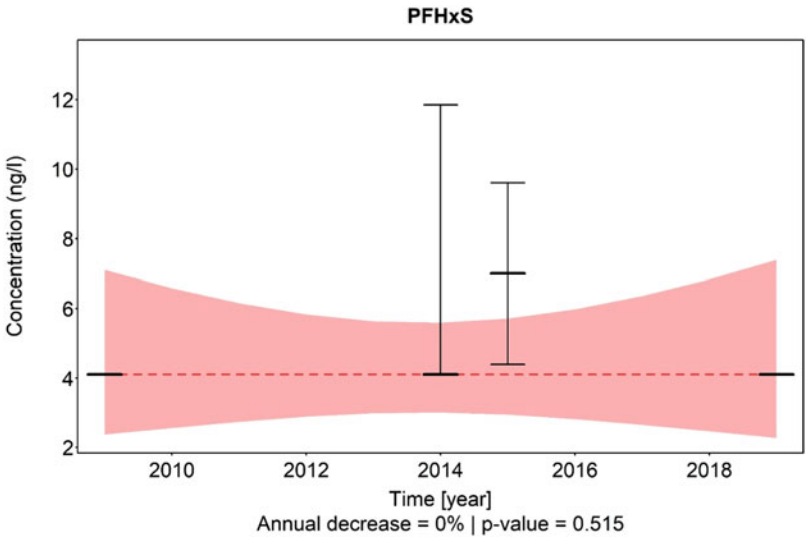

Fig. 48 Theil–Sen exponential trends of PFHxS concentrations in human milk (ng/L) in countries of the Eastern European Group (12 national pools, 8 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles. To replace left-censored values, values under LOQ were substituted by 1/sqrt(2) of the LOQ.)

3.3.5 Western European and Others Group (WEOG)

From 8 samples of 7 countries, 5 samples were below LOQ (<5.7 ng/L), three samples above (Netherlands, 2014: 8.6 ng/L; Sweden, 2019: 17.9 ng/L; Switzerland, 2016: 8.7 ng/L) (Fig. 56 in the appendix). No country participated repeatedly. Therefore, no temporal tendencies can be derived, neither in general between periods based on results of all countries, nor by combining data from all countries and use of the Theil–Sen method (Fig. 49) nor country-specific for countries with repeated participation.

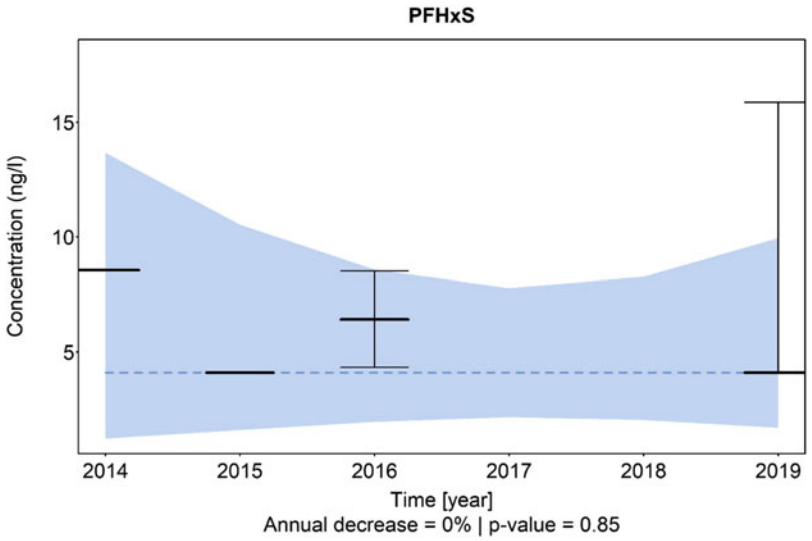

Fig. 49 Theil–Sen exponential trends of PFHxS concentrations in human milk (ng/L) in countries of the Western European Group and Others Group (8 national pools, 7 countries). (The shaded area shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles. To replace left-censored values, values under LOQ were substituted by 1/sqrt(2) of the LOQ.)

3.3.6 Worldwide

Due to the very high percentage of samples below LOQ, no temporal trends could be derived, neither by combining all samples and use of Theil–Sen method (Fig. 50) nor by combining all samples from countries with repeated participation and use of Theil–Sen method (Fig. 51).

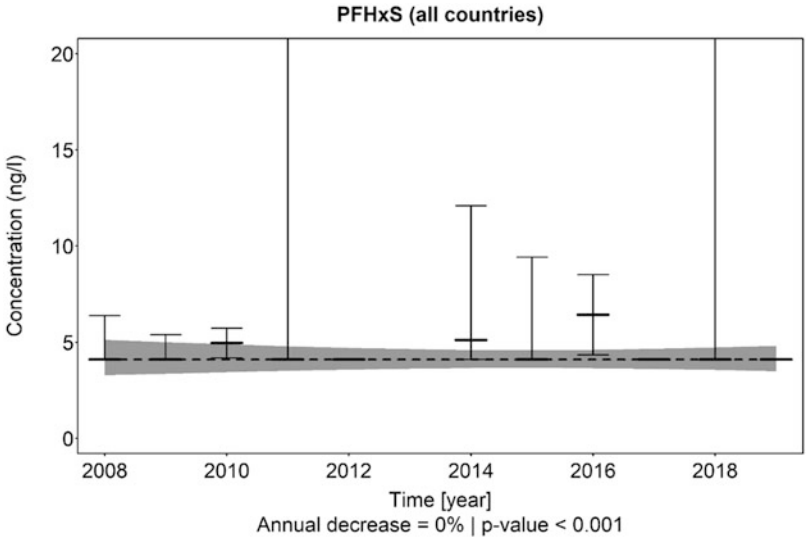

Fig. 50 Theil–Sen exponential trends of PFHxS concentrations in human milk (ng/L) worldwide using data from all countries (86 national pools, 59 countries). (The shaded are shows the 95% confidence interval of the trend; the thick black lines in the middle of the frequency distribution in a certain year show median concentrations in individual years, hinges and whiskers show ranges between fifth and 95th percentiles. To replace left-censored values, values under LOQ were substituted by 1/sqrt(2) of the LOQ.)

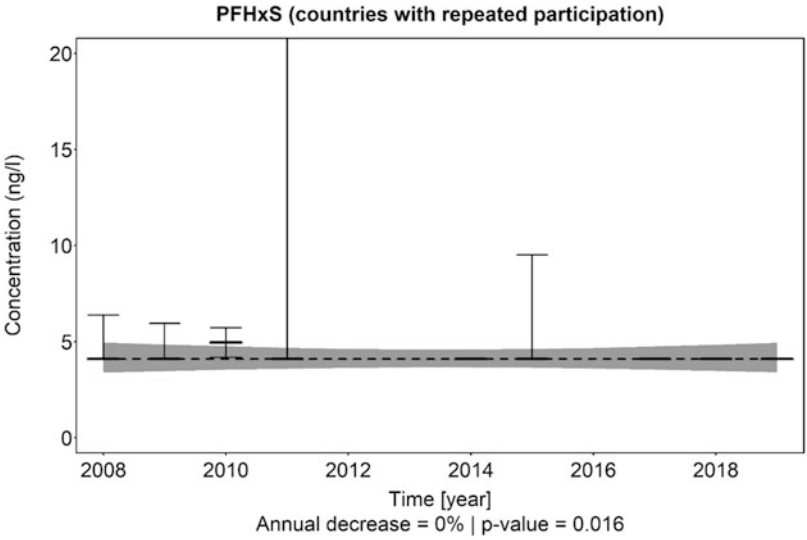

Fig. 51 Theil–Sen exponential trends of PFOA in human milk (ng/L) worldwide using data from countries with repeated participation (48 national pools, 24 countries)

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing Regional Support for the POPs Global Monitoring Plan Under the Stockholm Convention in the Africa, Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the UNEP/WHO global human milk survey, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk surveys, are greatly appreciated.

Data analysis was supported by the RECETOX Research Infrastructure (No LM2018121) and the CETOCOEN EXCELLENCE Teaming Phase 2 project financed by the European Union's Horizon 2020 (No 857560) and the European Structural and Investment Funds: (No CZ.02.1.01/0.0/0.0/17_043/0009632).

The authors express their gratitude to the National Coordinators of the WHO- and UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratories, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff.

Rolaf van Leeuwen, Gerald Moy, Angelika Tritscher, Hae Jung Yoon, Seongsoo Park, and Philippe Verger (Department of Food Safety and Zoonoses) are acknowledged for their coordinating support during their time at WHO.

The authors thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Jacqueline Alvarez, Haosong Jiao and Gamini Manuweera (United Nations Environment Programme, Economy Division, Chemicals and Health Branch) for their support and contributions to these surveys, furthermore Heidelore Fiedler for the conception and implementation of the GMP projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

Disclaimer The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions, policy, or views of the World Health Organization and the United Nations Environment Programme.

Appendix

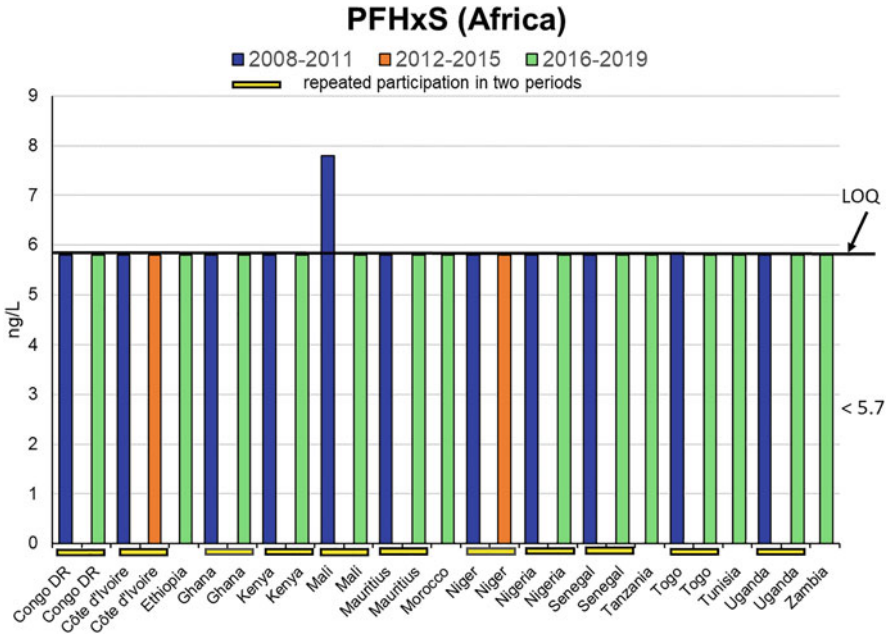

Fig. 52 Illustration of findings of PFHxS concentrations around the limit of quantification (LOQ) in human milk (ng/L) from African countries over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles)

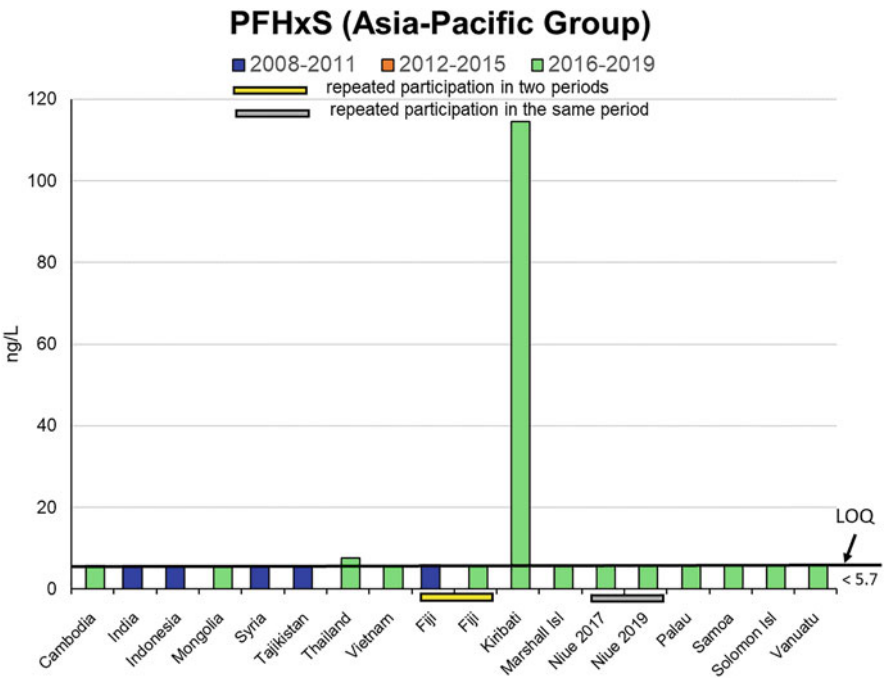

Fig. 53 Illustration of findings of PFHxS concentrations around the limit of quantification (LOQ) in human milk (ng/L) from countries of the Asia-Pacific Group over time (period 2008–2011 in blue, period 2012–2015 in orange [however, without samples in this period], and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles, with repeated participation in the same period by grey rectangles)

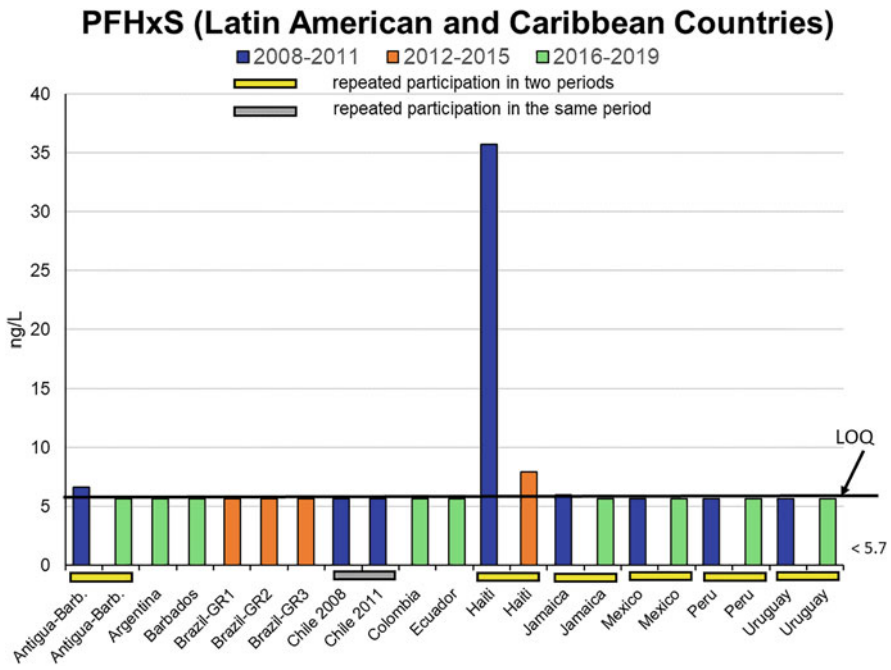

Fig. 54 Illustration of findings of PFHxS concentrations around the limit of quantification (LOQ) in human milk (ng/L) from Latin American and Caribbean countries over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles, with repeated participation in the same period by grey rectangles)

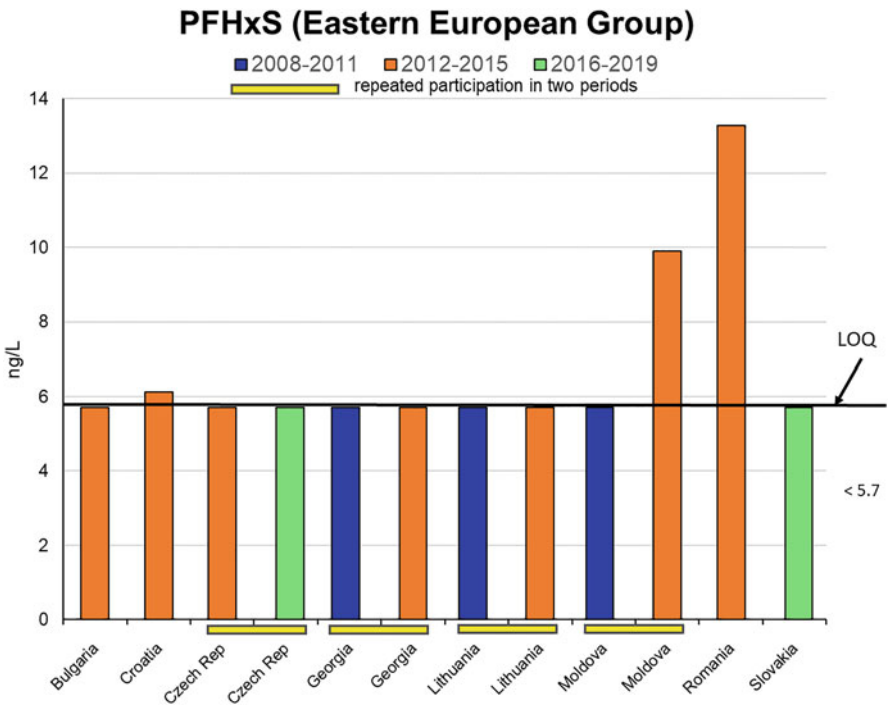

Fig. 55 Illustration of findings of PFHxS concentrations around the limit of quantification (LOQ) in human milk (ng/L) from countries of the Eastern European Group over time (period 2008–2011 in blue, period 2012–2015 in orange, and period 2016–2019 in green; countries with repeated participation in two of these periods marked by yellow rectangles)

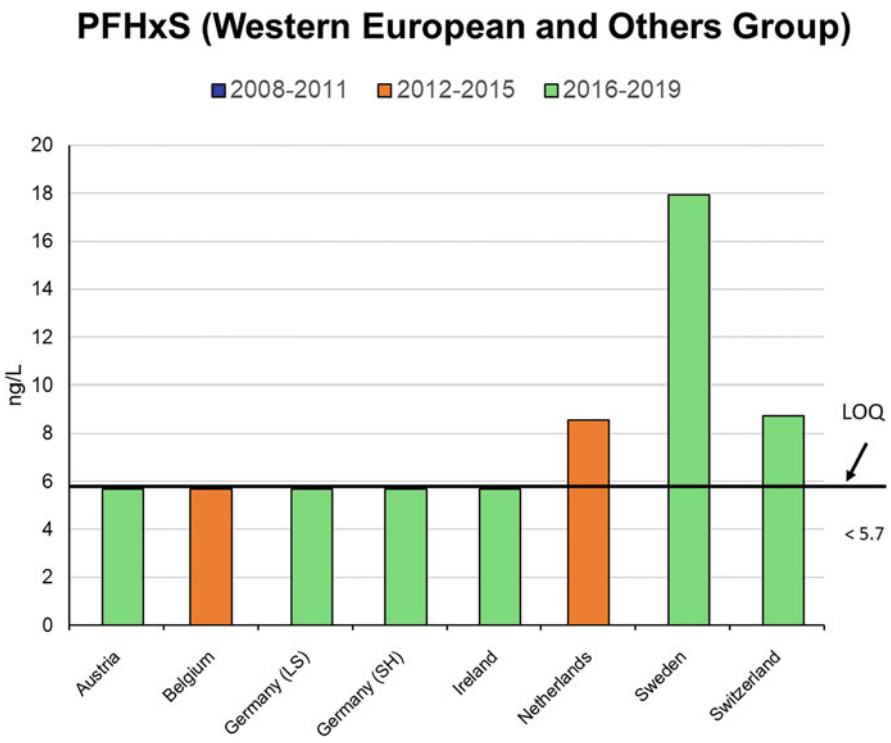

Fig. 56 Illustration of findings of PFHxS concentrations around the limit of quantification (LOQ) in human milk (ng/L) from countries of the Western European and Others Group over time (period 2008–2011 in blue [however, without samples in this period], period 2012–2015 in orange, and period 2016–2019 in green)

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Risk–Benefit Analysis for the Breastfed Infant Based on the WHO- and UNEP Human Milk Surveys for Dioxin-like Compounds

Martin van den Berg, Majorie B. M. van Duursen, Angelika Tritscher, Rainer Malisch, and Richard E. Peterson

Abstract

Dioxin-like compounds (DLC) are still present in human milk and this chapter describes a risk–benefit analysis based on decades of WHO global human milk surveys. At present there is no health-based guidance value (HBGV) available for the breastfed infant. Although formally these HBGVs have been set to protect human health for a lifetime exposure period, much of the underlying experimental data focus on the perinatal and/or childhood period. Therefore, it is justifiable to use these HBGVs for early life and shorter than lifetime exposures, e.g. breastfeeding. With this approach the present HBGVs for DLC were generally exceeded one order of magnitude or more in industrialized countries over the period 2000 to 2019. If HBGVs of 1 or 0.1 pg TEQ/kg/day are used to calculate toxicological acceptable levels for DLC in human milk, it can be estimated that such levels will not be reached before, respectively, 2030 or 2050. When the subtle adverse health effects of DLC in the breastfed infant reported in the 1990s

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_15

were compared with benefits of breastfeeding for the infant and mother, it is concluded that benefits grossly outweigh the potential adverse health. Therefore, it is concluded that the WHO has rightfully encouraged breastfeeding for the last decades.

Keywords

Human milk · Dioxin-like compounds · PCDD/PCDF ("dioxins") · PCB · Breastfeeding · Risk-benefit

1 Introduction

In the mid-1980s and early 1990s, the World Health Organization (WHO) coordinated two exposure studies on concentrations of polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) in human milk (WHO 1989; WHO 1996). After adoption of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2001, the WHO and the United Nations Environment Programme (UNEP) agreed to collaborate in joint studies starting in 2004 to support the implementation of this convention by assessing its effectiveness as required under its Article 16. Between 2000 and 2019, WHO and UNEP performed five global voluntary surveys to determine concentrations of POPs in human milk with participation of 82 countries (Malisch et al. 2023a).

As a result of these regular surveys, the present data provide significant and valuable information regarding regional aspects and time trends for a broad range of POPs, including dioxin-like compounds (DLC). Findings between 2000 and 2019 have been described in detail in various publications of compendium, including PCB and PCDD/PCDF in Part III (Malisch et al. 2023b). As such, this information can be used to estimate the possible health risks of some POPs that may be associated with breastfeeding but can also be used to estimate a possible reduction in future health risks. PCDD and PCDF were the first group of POPs that were monitored in these human milk surveys (WHO 1989; 1996). Temporal trends of PCB and PCDD/PCDF derived from repeated participation of countries between 1987 and 2019 have also been assessed in Part IV (Malisch et al. 2023c). Consequently, possible time trends and associated risks of these compounds for the neonate can already be determined as early as the 1980s and 1990s.

With increasing knowledge about the mechanism of action and toxicity of these PCDD and PCDF, there was a growing awareness within the scientific community that some PCB were structural analogs of DLC. These so-called dioxin-like PCB (DL-PCB) were found to have similar toxic symptoms as the more classic DLC, like 2,3,7,8-TCDD (Safe 1990; 1994). Based on these scientific insights, most leading toxicologists in the field agreed that for human risk assessment the group of DLC needed to be expanded with DL-PCB. A broad range of experimental studies has also established that most, if not all, toxicological effects of DLC were mediated by one receptor, the aryl hydrocarbon receptor (AhR). This knowledge led to the

concept of additivity for DLC. Although not perfect, it is still considered the most realistic way to describe mixture toxicity of these compounds (Birnbaum 1994).

Subsequently, it was globally accepted by regulatory authorities that human risk assessment for mixtures of these DLC should follow an additive approach. To support and globally harmonize the human risk assessment of DLC, the WHO initiated several expert meetings during the last decades. During these meetings (interim) toxic equivalency factors (TEF) were proposed to standardize the assessment for these compounds (Ahlborg et al. 1994; Van den Berg et al. 1998; Van den Berg et al. 2006). These WHO-TEF have now worldwide acceptance by regulatory authorities and are commonly used to determine toxic equivalency values (TEQ) of chlorinated DLC in, e.g., feed and food, environmental samples, or human matrices. Moreover, it has been brought forward that this TEF concept should also be expanded to POPs with a similar mechanism of action, e.g. brominated analogs of DLC. As a result, the brominated dioxins and dibenzofurans have more recently also been included in the WHO TEF model (Van den Berg et al. 2013).

2 Global Measurements and Time Trends

Although not all countries consistently participated in the WHO/UNEP-coordinated human milk surveys since the start of this program, the present database provides a unique opportunity to study the decrease in human exposure over time. In countries with a repeated participation in these surveys, the PCDD/PCDF concentrations in human milk decreased from a median level of 17 pg WHO_{2005}-PCDD/PCDF-TEQ/g lipid in the mid-1980s to 3 pg WHO_{2005}-PCDD/PCDF-TEQ/g lipid in the 2016–2019 period (WHO 1989; Malisch et al. 2023c). Undoubtedly, this significant and worldwide reduction of more than 80% during a 30-year period was caused by rigorous regulatory actions that started in the 1990s. These measures significantly reduced the emissions of PCDD and PCDF from combustion processes, e.g. from municipal incinerators, as well as their reduction in a variety of chemical products. As could be expected, this reduction was also reflected in human milk contamination with these compounds and should no doubt be considered as a success story for global regulatory actions.

With growing awareness of the dioxin-like properties of some PCB, the WHO human milk surveys also offer unique insights into the decrease of levels over time as the DL-PCB were included in the analyses from the beginning of the 1990s. Overall, the decreasing time trend of DL-PCB in human milk followed the same declining trend as PCDD and PCDF (Malisch et al. 2023c). Unlike unintentionally formed PCDD and PCDF, PCB were produced commercially for open applications, e.g. in paints and sealants, and in closed applications like transformers and capacitors as cooling fluids. In the case of PCB, it is important to realize that this observed reduction of PCB in human milk was caused by severe restrictions on commercial production of these compounds that already started in the 1970s and 1980s.

Due to the similar mechanism of action of DL-PCB compared with PCDD and PCDF, the WHO assigned TEF values for these congeners since the 1990s (Ahlborg

et al. 1994; Van den Berg et al. 1998). However, with growing scientific insights into the dioxin-like properties of these PCB, some WHO-TEF values changed over time (Van den Berg et al. 2006). To provide a consistent insight into time trends, human milk levels of PCDD, PCDF, *and* DL-PCB in this assessment have all been expressed as total TEQ (WHO-PCDD/PCDF-PCB-TEQ) using the TEF as agreed upon in the 2005 WHO expert meeting (Van den Berg et al. 2006). It was observed in 52 countries during the 2000–2010 period of WHO surveys that DL-PCB represented approximately 30 to 50% of the total WHO_{2005}-TEQ in human milk, albeit with some noticeable regional differences (Van den Berg et al. 2017). In 82 countries participating between 2000 and 2019, DL-PCB contributed between 8% and 62% (median: 33%) to the total WHO_{2005}-TEQ (Malisch et al. 2023b). In most industrialized countries, a decline of 80% or more in total WHO_{2005}-TEQ can be observed over the last 25 years. The highest levels of total WHO_{2005}-TEQ were observed in Western Europe with a median level of nearly 30 pg WHO_{2005}-TEQ/g lipid in the 1990s (countries with repeated participation; range about 20 WHO_{2005}-TEQpg/g lipid to 35 pg WHO_{2005}-TEQ/g lipid) decreasing to approximately 5 pg WO_{2005}-TEQ/g lipid in the period 2016–2019.

In view of the presence of these dioxin-like compounds in human milk and their decreasing time trend, the major issue addressed in this chapter is the possible health risk to the breastfed infant via breastfeeding. Such possible health risk has been assessed earlier with the WHO human milk surveys performed in the period 2000 to 2010 (Van den Berg et al. 2017). In that study, it was concluded that global TEQ levels in human milk were still above the levels that would be toxicologically acceptable for the breastfed infant. Nevertheless, it was also concluded in the previous assessment that the benefits of breastfeeding by far outweighed the possible health risks of these DLC for the breastfed infant. In this chapter, this earlier risk–benefit analysis is revisited against the decreasing time trend of DLC over the last 25 years.

3 Most Sensitive Endpoint for the Breastfed Infant

When determining possible or potential health risks of DLC for the breastfed infant, a major uncertainty is the contribution of prenatal versus postnatal exposure. These different exposure routes cannot easily be separated under normal perinatal conditions. In addition, it has been established that prenatal exposure is also highly relevant for the developmental toxicity of these compounds (Peterson et al. 1993). Moreover, at present there is no health-based guidance value (HBGV) available for the infant in relation to lactational exposure that would distinguish an effect between pre- and postnatal exposure. However, many regulatory agencies have set HBGVs for lifetime exposure situations and DLC, and a number of these are presented in Table 1 (ATSDR 1998; WHO 2000; US-EPA 2010; EFSA 2018).

Irrespective of whether a HBGV for DLC is applied based on 2,3,7,8-TCDD or on total TEQ, it is clear that global levels in human milk almost always exceed existing guidance values of exposure (see Table 1). These exceedances of HBGVs

Table 1 Health-Based Guidance Values (HBGVs) determined by various regulatory authorities with their underlying health effects in animals and humans with an estimated acceptable HBGV in WHO_{2005}-TEQ in human milk

Organization	Health-based guidance value (HBGV)		Exceedance HBGV[a,b] (2015–2020)	Associated HBGV milk level in pg TEQ/g lipid[c]	Health endpoints used in offspring
WHO (2000)	TDI	1–4 pg TEQ/kg bw/day	4–14 x	0.2–0.9	Offspring monkey, mouse, rat: Decreased sperm count, genital malformations, immune suppression, neurobehavioral effects after perinatal exposure
JECFA (2002)	TMI	70 pg TEQ/kg bw/month	6 x	0.5	Male rat: Reproductive tract deficits after prenatal exposure
US-EPA (2010)	RfD[d]	0.7 pg TEQ/kg bw/day	19 x	0.2	Human: Decrease sperm count and motility after childhood exposure
ATSDR (1998)	$MRL_{subchronic}$	20 pg TCDD/ kg bw/day	0.7 x	4.6	Weanling Guinea pig: Immunosuppression after 3 months exposure
ATSDR (1998)	$MRL_{chronic}$	1 pg TCDD/kg bw/day	14 x	0.2	Offspring rhesus monkey: Neurobehavioral effects after perinatal exposure
EFSA (2018)	TWI	2 pg TEQ/kg bw/week	47 x	0.07	Human: Decreased sperm concentration after childhood and perinatal exposure

[a]Based on recent median exposure levels of 3 pg TEQ/g lipid
[b]Based on 3.5% lipid weight in human milk and infant consumption of 125 g milk/kg bw/day, set a 4.5 g lipid/kg bw/d
[c]HBGV derived level pg TEQ/g lipid (HBGV in pg TEQ/kg bw/day)/4.375 g lipid/kg bw/day
[d]Reference dose

are generally a factor 10 or more, based on the medians of samples from more heavily industrialized countries, e.g., in Western Europe, from the period 2000 to 2019. The only exception is found for the ATSDR assigned semi-chronic MRL that was established before 2000 (ATSDR 1998). In this respect, it should be noted that significant additional insights into health effects of dioxin-like compounds were obtained from 2000 onward (US-EPA 2010; EFSA 2018).

Formally, these HBGVs have usually been defined and set to assure a lifetime daily exposure without human health risk. Therefore, it has frequently been argued that these HBGVs should not be applied for the breastfed infant situation, as lactational exposure is usually limited to a period of approximately three months to two years. Though, if the underlying experimental studies that serve as a point of departure for these HBGVs are given a more detailed look, it can be observed that their exposure time-period was significantly shorter than a full lifespan of the animals. Moreover, several regulatory agencies derived their HBGVs from developmental effects in animal and epidemiological studies. These originated from either prenatal, perinatal, or childhood exposure to DLC, thereby often including the lactational period, as illustrated below.

In the total daily intake (TDI) determination of 1 to 4 pg TEQ/kg bw day, the WHO used experimental studies with offspring of monkeys, rats, and mice that were perinatally exposed, as can be seen in Table 4 from their publication (WHO 2000). Subsequently, the 57th joint FAO/JECFA meeting also considered developmental effects on the male reproductive tract in rats after prenatal exposure as the most sensitive endpoint to derive a tolerable monthly intake (TMI) value (JECFA 2002). Moreover, the ATSDR established MRL for both semi-chronic and chronic exposure scenarios. For the semi-chronic exposure scenario, immunosuppression in weanling guinea pigs was used after a period of three months exposure, while for chronic exposure the perinatal exposure situation in Rhesus monkeys was used (ATSDR 1998). Most recently, EFSA derived a human based HBGV from one epidemiological study with exposure during childhood, which resulted in decreased sperm concentrations at adult age as the most sensitive human endpoint. Based on the observed NOAEL in that particular study EFSA also calculated that an amount of 5.9 pg TEQ/g lipid in human milk was associated with this NOAEL later in life (EFSA 2018). The above information is summarized in Table 1.

Considering the endpoints that were used to derive HBGVs, it can be concluded that generally toxicological and epidemiological studies were used with perinatal animal or childhood exposure to DLC, thus including the lactational period. It is well-established that developmental effects of DLC originating from early lifetime exposure are by far the most sensitive endpoints in all vertebrates (Peterson et al. 1993; WHO 2000; US-EPA 2010). Therefore, it can be concluded that risks from lactational exposure is covered by established HBGVs and that HBGVs for lifetime exposure may provide the highest protection, including vulnerable populations, such as the neonate and developing child. Noteworthy, these HBGVs were then "upgraded" by regulatory authorities to be used for the lifetime exposure situation either for TCDD or TEQ. In our opinion, it is justifiable to use established HBGVs for early life and shorter than lifetime exposure, e.g. breastfeeding.

Taken together, the results from these experimental studies used to derive HBGVs already suggest a further decrease of DLC in human milk since the 1990s. Moreover, epidemiological studies in the 1990s with breastfed infants from The Netherlands already showed an association between thyroid hormone level changes, immunological and (neuro)developmental effects with increasing levels of DLC (Pluim et al. 1993; Koopman-Esseboom et al. 1994; 1996; Weisglas-Kuperus et al. 1995, 2000, 2004). Many of these studies were performed at the end of the previous century when TEQ levels in human milk were at least one order of magnitude higher than in the most recent decade. The question arises whether such associations could still be found with current levels of DLC in human milk and maternal blood. With this in mind, it remains unclear if the effects found in the Dutch studies at that time were (in part) caused by prenatal exposure or postnatal human milk exposure. For DLC, animal studies clearly support a significant role for prenatal exposure for a range of sensitive toxicological effects of DLC (Peterson et al. 1993). The importance of prenatal exposure is also supported by more recent human studies. For example, a mother–child cohort study with children from Greece and Spain suggests a decrease in anogenital distance (AGD) in young boys with increasing maternal blood levels of DLC (Vafeiadi et al. 2013). In this study, median TEQ levels in blood declined after birth (52.3 ± 20.7 and 49.7 ± 26.7 pg TEQ/g lipid in newborns and young children, respectively). Moreover, the duration of breastfeeding was short (median 2 months) and was not associated with AGD, possibly suggesting a prevailing prenatal effect of DLC on AGD.

When considering all underlying toxicological and epidemiological information, there are good arguments to use established HBGVs (see Table 1) also for the breastfed infant and not only for lifetime exposure. Here, the strongest supporting argument would be the fact that many, if not all, underlying studies for these HBGVs are addressing a relatively short early lifetime exposure situation, including the lactational period, instead of a full lifespan. In Table 1, the calculated "acceptable" human milk levels of DLC associated with different HBGVs are presented. With the current state of knowledge, these toxicological "acceptable" levels are estimated to be in the range of 0.1 to 1 pg WHO_{2005}-TEQ/g lipid in human milk. This estimated "acceptable" range of TEQ in human milk is slightly lower than that calculated by EFSA (2018), which is 5.9 pg TEQ/g lipid. When reviewing this modest difference between EFSA's calculated safe human milk level and our "acceptable" TEQ range it should be recognized that the EFSA calculation is based only on one human study, while our estimated "acceptable" range contains a multiplicity of experimental studies.

When these projected "acceptable" levels are evaluated with the average decreases in some European countries with the highest concentrations of DLC in human milk, it can be estimated when "acceptable" levels will be reached in the foreseeable future. For a decline extrapolated with a HBGV of 1 pg WHO_{2005}-TEQ/g lipid, a toxicologically acceptable level may be reached around 2030, while for 0.1 WHO_{2005}-TEQ/g lipid such a level would not be reached before 2050. These situations are illustrated in Fig. 1. In either case it will still take decades from now to attain toxicologically "acceptable" levels of DLC in human milk. This estimation

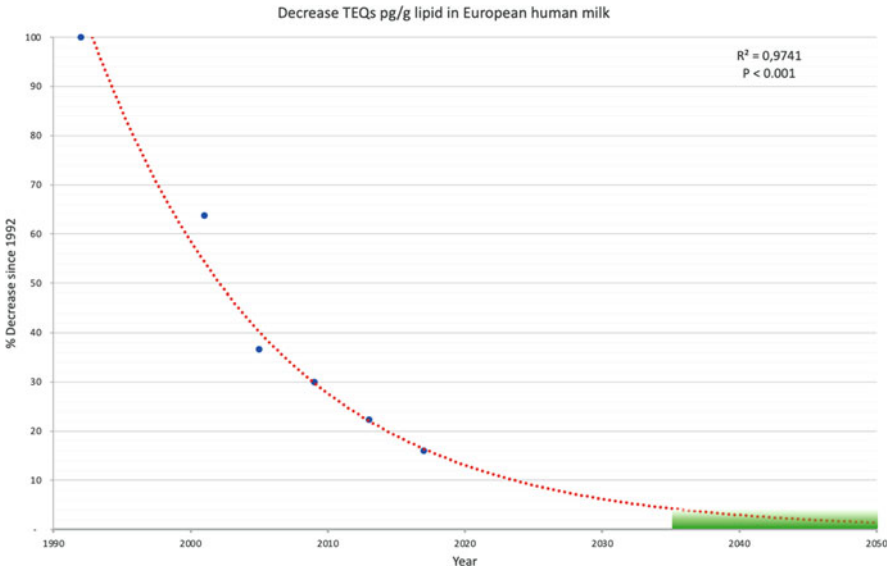

Fig. 1 Average percent decline in human milk WHO_{2005}-TEQ levels* in European countries** since 1992 and expected levels based on health-based guidance levels of 0.1 to 1 pg TEQ/g lipid (see Table 1). * Average for these countries in 1992 set at 25 pg WHO_{2005}-TEQ/g lipid and 100% with the green bar indicating an HBGV based projected decline of 0.4 to 4% of the 1992 level. ** Including The Netherlands, Lithuania, Belgium, Germany, Norway, Slovak Rep., Finland, Czech Rep., Croatia

clearly points out that (further) remedial actions are still needed, especially if the goal is to reach these "acceptable" levels for the breastfed infant sooner.

When considering the potential adverse health effects of DLC in human milk for the infant, it is of utmost importance to evaluate these in conjunction with the benefits of breastfeeding. There is no doubt, that a wide array of epidemiological studies convincingly showed the important health benefits of breastfeeding for both the infant and mother (Horta et al. 2007; Ip et al. 2007; James et al. 2009; Victora et al. 2016; Del Ciampo and Del Ciampo 2018). It is beyond the scope of this chapter to review and discuss all of these, but results of these studies are summarized in Table 2. As an indication, a recent study using meta-analyses of benefits from breastfeeding calculated that it may annually prevent 823,000 deaths in children younger than 5 years and 20,000 maternal deaths from, e.g., breast cancer (Victora et al. 2016). In contrast, the adverse health effects observed at concentrations of DLC in human milk that were present in the 1990s were considered limited from a clinical point of view and often transient (Lapillonne et al. 2021), e.g. thyroid hormone changes and liver functions (Ten Tusscher and Koppe 2004). Nevertheless, it should also be recognized that some of these subtle effects on, e.g., cognitive functions and the immune system were persistent beyond the prenatal and childhood period (Ten Tusscher et al. 2003, 2014). At present, the impact of these sustained effects later in life is unclear but should not be neglected due to lack of knowledge.

Table 2 General overview for the observed benefits* of breastfeeding for the infant and mother (Van den Berg et al. 2017)

Benefits for the infant:	**Benefits for the mother**:
• Optimal nutrition • Strong bonding with mother • Safe milk • Enhanced immune system • Reduced risk of acute otitis media, gastroenteritis, lower respiratory tract infections, and asthma • Protection against allergies • Correct development of jaw and teeth • Association with higher IQ/school performance • Reduced risk of chronic diseases, e.g. obesity, diabetes, heart disease, hypertension, hypercholesterolemia, childhood leukemia • Reduced risk of sudden infant death syndrome • Reduced risk of overall morbidity and mortality	• Strong bonding with infant • Increased energy expenditure, faster return to prepregnancy weight • Faster shrinking of the uterus • Reduced postpartum bleeding and delay menstrual cycle • Decreased risk of chronic diseases, e.g. breast, and ovarian cancer, diabetes • Improved bone density, decreased risk hip fracture • Decreased risk of postpartum depression • Enhanced self-esteem in the maternal role Time and money saved from preparing and not buying formula, less medical expenses

Thus, when comparing the significant beneficiary health effects of breastfeeding with these effects associated with DLC in human milk and maternal blood in the 1990s, the health benefits for infant and mother still grossly outweighed these potential adverse subtle health effects of these compounds. In addition, the question can be raised if reported adverse health effects of DLC in the 1990s were (partly) attributable to prenatal exposure. Furthermore, it is unknown whether these adverse effects would still be found with the present DLC levels in human milk, as levels of DLCs in breast milk are now at least one order of magnitude lower. Based on the above arguments and evaluation it can be concluded that the WHO has rightfully encouraged breastfeeding globally for the last decades and should continue to do so (WHO 2009).

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Part V

Summary, Conclusions and Outlook

Overall Conclusions and Key Messages of the WHO/UNEP-Coordinated Human Milk Studies on Persistent Organic Pollutants

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_16

Abstract

Building on the two rounds of exposure studies with human milk coordinated by the World Health Organization (WHO) in the mid-1980s and 1990s on polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF), five expanded studies on persistent organic pollutants (POPs) were performed between 2000 and 2019. After the adoption of the Stockholm Convention on POPs (the Convention) in 2001, WHO and the United Nations Environment Programme (UNEP) collaborated in joint studies starting in 2004. The collaboration aimed at provision of POPs data for human milk as a core matrix under the Global Monitoring Plan (GMP) to assess the effectiveness of the Convention as required under Article 16. Over time, the number of analytes in the studies expanded from the initial 12 POPs targeted by the Convention for elimination or reduction to the 30 POPs covered under the Stockholm Convention and two other POPs proposed for listing as of 2019. Many of these chemicals have numerous congeners, homologous groups, isomeric forms, and transformation products, which significantly extends the number of recommended analytes.

In the studies between 2000 and 2019, 82 countries from all five United Nations regions participated, of which 50 countries participated in more than one study. For the human milk samples of the 2016–2019 period, results are available for the full set of 32 POPs of interest for the Convention until 2019: (i) the 26 POPs listed by the start of the study in 2016; (ii) decabromodiphenyl ether [BDE-209] and short-chain chlorinated paraffins [SCCP] as listed in 2017; (3) dicofol and perfluorooctanoic acid [PFOA] as listed in 2019; (4) medium-chain chlorinated paraffins [MCCP] and perfluorohexane sulfonic acid [PFHxS] as proposed for listing. This is a unique characteristic among the core matrices under the GMP.

Four key messages can be derived:

(i). These studies are an **efficient and effective tool with global coverage as key contributor to the GMP**. After collection of a large number of individual samples (usually 50) fulfilling protocol criteria, pooled samples are prepared using equal aliquots of individual samples (physical averaging) and are considered to be representative for a country, subregion or subpopulation at the time of the sampling. The analysis of pooled representative human milk samples by dedicated Reference Laboratories meeting rigorous quality criteria contributes to reliability and comparability and reduces uncertainty of the analytical results. Additionally, this concept is very cost-effective.

(ii). These studies can be used for **regional differentiation** based on concentrations of individual POPs between and within the five UN Regional Groups (African Group, Asia-Pacific Group, Eastern European Group, Group of Latin American and Caribbean Countries; Western European and Others Group). For some POPs, a wide range of

concentrations with up to three orders of magnitude between lower and upper concentrations was found, even for countries in the same UN region. Some countries had levels within the usual range for most POPs, but high concentrations for certain POPs. Findings of concentrations in the upper third of the frequency distribution may motivate targeted follow-up studies rather than if the observed level of a POP is found in the lower third of frequency distribution. However, the concentration of a POP has also to be seen in context of the sampling period and the history and pattern of use of the POPs in each country. Therefore, results are not intended for ranking of individual countries but rather to distinguish broader patterns.

(iii). These studies can provide an **assessment of time trends**, as possible sources of variation were minimized by the survey concepts building on two factors (sampling design; analysis of the pooled samples by dedicated Reference Laboratories). The estimation of time trends based on comparison of median or mean concentrations in UN Regional Groups over the five surveys in five equal four-year periods between 2000 and 2019 provides a first orientation. However, the variation of the number of countries participating in a UN Regional Group in a certain period can influence the median or mean concentrations. Thus, it is more prudent to only use results of countries with repeated participation in these studies for drawing conclusions on temporal trends.

The reduction rates in countries should be seen in context with the concentration range: A differentiation of high levels and those in the range of the background contamination is meaningful. If high levels are found, sources might be detected which could be eliminated. This can lead to significant decrease rates over the following years. However, if low background levels are reported, no specific sources can be detected. Other factors for exposure, e.g. the contamination of feed and food by air via long-range transport and subsequent bioaccumulation, cannot be influenced locally.

However, only very few time points from most individual countries for most POPs of interest are available, which prevents the derivation of statistically significant temporal trends in these cases. Yet, the existing data can indicate decreasing or increasing tendencies in POP concentrations in these countries. Furthermore, pooling of data in regions allows to derive statistically significant time trends in the UN Regional Groups and globally. Global overall time trends using the data from countries with repeated participation were calculated by the Theil–Sen method. Regarding the median levels of the five UN Regional Groups, a decrease per 10 years by 58% was found for DDT, by 84% for beta-HCH, by 57% for HCB, by 32% for PBDE, by 48% for PFOS, by 70% for PCB, and by 48% for PCDD and PCDF (expressed as toxic equivalents). In contrast, the concentrations of chlorinated paraffins (CP) as "emerging POPs" showed increasing tendencies in some UN Regional Groups. On a global level, a statistically significant increase of total CP (total CP content including SCCP [listed in

the Convention in 2017] and MCCP [proposed to be listed]) concentrations in human milk of 30% over 10 years was found.

(iv). The studies can provide the basis for discussion of the **relative importance ("ranking") of the quantitative occurrence of POPs**. This, however, requires a differentiation between two subgroups of lipophilic substances ([i] dioxin-like compounds, to be determined in the pg/g [=ng/kg] range, and [ii] non-dioxin-like chlorinated and brominated POPs, to be determined in the ng/g [=μg/kg] range; both groups reported on lipid base) and the more polar perfluorinated alkyl substances (PFAS); reported on product base [as pg/g fresh weight] or on volume base [ng/L]. For this purpose, results for the complete set of the 32 POPs of interest for the 2016–2019 period were considered.

By far, the highest concentrations of lipophilic substances were found for DDT (expressed as "DDT complex": sum of all detected analytes, calculated as DDT; maximum: 7100 ng/g lipid; median: 125 ng/g lipid) and for chlorinated paraffins (total CP content; maximum: 700 total CP/g lipid; median: 116 ng total CP/g lipid). PCB was next in the ranking and had on average an order of magnitude lower concentrations than the average of the total CP concentrations.

The high CP concentrations were caused predominantly by MCCP. If the pooled samples from mothers without any known major contamination source nearby showed a high level of CP, some individual samples (e.g. from local population close to emission sources, as a result of exposure to consumer products or from the domestic environment) might even have significantly higher levels. The lactational intake of SCCP and MCCP of the breastfed infant in the microgram scale resulting from the mothers' dietary and environmental background exposure should therefore motivate targeted follow-up studies and further measures to reduce exposure (including in the case of MCCP, regulatory efforts, e.g. restriction in products).

Further, due to observed levels, targeted research should look at the balance among potential adverse effects against positive health aspects for the breastfed infants for three groups of POPs (dioxin-like compounds; non-dioxin-like chlorinated and brominated POPs; PFAS) regarding potentially needed updates of the WHO guidance.

As an overall conclusion, the seven rounds of WHO/UNEP human milk exposure studies are the largest global survey on human tissues with a harmonized protocol spanning over the longest time period and carried out in a uniform format. Thus, these rounds are an effective tool to obtain reliable and comparable data sets on this core matrix and a key contributor to the GMP. A comprehensive set of global data covering all POPs targeted by the Stockholm Convention, in all UN Regional Groups, and timelines covering a span of up to three decades allows to evaluate data from various perspectives. A widened three-dimensional view is necessary to discuss results and can be performed using the three pillars for assessments of the comprehensive data set, namely: analytes of

interest; regional aspects; time trends. This can identify possible problems for future targeted studies and interventions at the country, regional, or global level. Long-term trends give an indication of the effectiveness of measures to eliminate or reduce specific POPs. The consideration of countries with repeated participation in these studies provides the best possible database for the evaluation of temporal trends. The continuation of these exposure studies is important for securing sufficient data for reliable time trend assessments in the future. Therefore, it is highly recommended to continue this monitoring effort, particularly for POPs that are of public health concern.

Keywords

WHO/UNEP-coordinated exposure studies · Human milk · Persistent organic pollutant (POP) · UN Regional Groups · Global · Effective tool · Key contributor to Global Monitoring Plan (GMP) · Regional differentiation · Time trend · Relative importance of chemicals · Stockholm Convention on POPs

1 Introduction

In Part I of this compendium on WHO/UNEP-coordinated exposure studies on human milk it was shown that human milk has served for many decades as a suitable matrix to assess the overall exposure of the general population to persistent organic pollutants (POPs) (Fürst 2023). In the mid-1980s and early 1990s, the World Health Organization (WHO) coordinated two exposure studies on concentrations of polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) in human milk. After the adoption of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2001, WHO and the United Nations Environment Programme (UNEP) agreed to collaborate in joint studies starting in 2004 to support the implementation of the Convention by assessing its effectiveness as required under its Article 16. Between 2000 and 2019, WHO and UNEP performed five global studies on concentrations of POPs in human milk with the participation of 82 countries, 50 of them participating in more than one study (Malisch et al. 2023a). The countries were allocated to one of the five UN Regional Groups, namely, African Group, Asia and Pacific Group, Latin American and Caribbean Group, Eastern European Group, and Western European and Others Group (Table 1).

An overview of the Stockholm Convention, the Global Monitoring Plan (GMP) and its implementation by regional and global monitoring reports is given by (Šebková 2023). Human milk is a core matrix under the GMP. The objective of human biomonitoring within the GMP, which includes the WHO- and/or UNEP-coordinated human milk studies, is to identify temporal and, as appropriate, spatial trends in levels of POPs in humans to evaluate the effectiveness of the Convention.

From 2001 until 2019, the scope of POPs listed by the Stockholm Convention increased from 12 to 30 chemicals (28 chlorinated or brominated, 2 perfluorinated

Table 1 Countries from the five United Nations Regional Groups (the African Group, the Asia-Pacific Group, the Group of Latin America and the Caribbean, the Eastern European Group, and the Western European and Others Group) participating between 2000 and 2019 in WHO/UNEP-coordinated exposure studies *[Note that Cyprus is included in the Asia-Pacific Group and Australia, Israel, New Zealand, and USA are included in the "Western European and Others Group"]*

African Group	2000-2003	2004-2007	2008-2011	2012-2015	2016-2019
Congo (DR)			x		x
Côte d'Ivoire			x	x	
Djibouti			x		
Egypt	x				x
Ethiopia				x	x
Ghana			x		x
Kenya			x		x
Mali			x		x
Mauritius			x		x
Morocco					x
Niger			x	x	
Nigeria			x		x
Senegal			x		x
Sudan		x			
Tanzania					x
Togo			x		x
Tunisia					x
Uganda			x		x
Zambia					x

Asia-Pacific Group	2000-2003	2004-2007	2008-2011	2012-2015	2016-2019
Asia subgroup					
Cambodia					x
Cyprus		x			
Hong Kong SAR, China	x		x		
India			x		
Indonesia			x		
Korea (Rep.)			x		
Mongolia					x
Philippines	x				
Syria			x		
Tajikistan			x		
Thailand					x
Vietnam					x
Pacific Islands subgroup					
Fiji	x	x	x		x
Kiribati		x	x		x
Marshall Islands			x		x
Niue			x		x
Palau			x		x
Samoa			x		x
Solomon Islands			x		x
Tonga			x		
Tuvalu			x		
Vanuatu					x

Group of Latin America and the Caribbean	2000-2003	2004-2007	2008-2011	2012-2015	2016-2019
Antigua-Barbuda			x		x
Argentina					x
Barbados			x		x
Brazil	x			x	
Chile			2 x		
Colombia					x
Cuba			x		
Ecuador					x
Haiti		x	x	x	
Jamaica			x		x
Mexico			x		x
Peru			x		x
Suriname				x	
Uruguay			x		x

Eastern European Group	2000-2003	2004-2007	2008-2011	2012-2015	2016-2019
Bulgaria	x			x	
Croatia	x			x	
Czech Rep.	x	x		x	x
Georgia			x	x	
Hungary	x	x			
Lithuania			x	x	
Moldova			x	x	
Romania	x			x	
Russia	x				
Slovak Rep.	x	x			x
Ukraine	x				

Western European and Others Group	2000-2003	2004-2007	2008-2011	2012-2015	2016-2019
Australia	x		x	x	
Austria					x
Belgium	x	x	x	x	
Finland	x	x			
Germany	x				x
Ireland	x		x		x
Israel				x	
Italy	x				
Luxembourg	x	x			
Netherlands	x			x	
New Zealand	x		x		
Norway	x	x			
Spain	x				
Sweden	x	x			x
Switzerland			x		x
USA	x				

substances). Many of these chemicals have numerous congeners, homologous groups, isomeric forms, and transformation products, which significantly extends the number of recommended analytes. Furthermore, two POPs proposed for listing in 2019 were of interest (medium-chain chlorinated paraffins [MCCP] and perfluorohexane sulfonic acid [PFHxS]) and included on a preliminary basis. Thus, over time the surveys covered an increasing number of analytes of interest (UNEP, 2019). For the human milk samples of the 2016–2019 period, results for the complete set of 32 POPs of interest for the Convention until 2019 are available (including decabromodiphenylether [BDE-209] and short-chain chlorinated paraffins [SCCP] as listed in 2017, dicofol and perfluorooctanoic acid [PFOA] as listed in 2019, PFHxS as proposed for listing in 2019 and adopted in 2022, and MCCP as under review by the POPs Review Committee) (Table 2)—a unique characteristic among the core matrices.

The data are publicly available in the Data Warehouse of the Stockholm Convention Global Monitoring Plan (GMP DWH) (GMP DWH 2020).

This chapter presents summarizing conclusions of the WHO/UNEP-coordinated exposure studies and key messages.

2 Efficient and Effective Tool with Global Coverage as Key Contributor to the Global Monitoring Plan (GMP)

Generally, the concept of the WHO/UNEP-coordinated exposure studies of human milk has four basic elements (Malisch et al. 2023a):

(i). Collection of a large number of individual samples from mothers guided by the criteria of the standardized WHO/UNEP protocols, under the supervision of a National Coordinator in each country
(ii). Preparation of pooled (physically averaged) samples that are considered to represent the average levels of POPs in human milk for a country or subpopulation/region of that country at the time of sampling, using equal aliquots of individual samples
(iii). Analysis of these pooled samples in designated Reference Laboratories to achieve a high degree of reliability and comparability of the analytical results
(iv). Repeated participation of countries allowing conclusions on temporal trends

This concept has a number of advantages.

2.1 Possibility to Determine the Complete Set of Analytes of Interest

As no multi-method exists allowing to determine all POPs of interest to the Stockholm Convention by one single method, various analytical methods have to be applied. Due to the relatively high fat content of human milk and the large volume

Table 2 Chemicals and analytes recommended for analysis of the core matrix "human milk" (as of 2019, 30 listed POPs and two chemicals of interest proposed for listing)

	Initial POPs (2001)	Recommended analytes for human milk
1	Aldrin	Aldrin
2	Chlordane	cis- and trans-chlordane; cis- and trans-nonachlor, oxychlordane
3	DDT	4,4′-DDT, 2,4′-DDT, 4,4′-DDE, 2,4′-DDE, 4,4′-DDD, 2,4′-DDD
4	Dieldrin	Dieldrin
5	Endrin	Endrin
6	HCB	HCB
7	Heptachlor	Heptachlor and heptachlorepoxide
8	Mirex	Mirex
9	PCB	$\sum PCB_6$ (6 congeners): 28, 52, 101, 138, 153, and 180
		PCB with TEFs* (12 congeners): 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189
10	PCDD	2,3,7,8-substituted PCDD (7 congeners)
11	PCDF	2,3,7,8-substituted PCDF (10 congeners)
12	Toxaphene	Congeners P26, P50, P62
	* PCB with TEFs (Toxic Equivalency Factors) assigned by WHO in 1998	
	POPs listed at COP-4 (2009)	
13	Chlordecone	Chlordecone
14	Alpha-hexachlorocyclohexane	alpha-HCH
15	Beta-hexachlorocyclohexane	beta-HCH
16	Gamma-hexachlorocyclohexane	gamma-HCH
17	Hexabromobiphenyl	PBB 153
18	Pentachlorobenzene	PeCBz
19	Tetra- and pentabromodiphenyl ether	PBDE 47, 99; optional: PBDE 100
20	Hexa- and heptabromodiphenyl ether	PBDE 153, 154, 175/183 (co-eluting)
21	Perfluorooctane sulfonic acid	PFOS (linear and branched isomers)
	POPs listed at COP-5 (2011)	
22	Endosulfan	alpha-, beta-endosulfan; endosulfan sulfate
	POPs listed at COP-6 (2013)	
23	Hexabromocyclododecane	alpha-, beta-, gamma-HBCD
	POPs listed at COP-7 (2015	
24	Hexachlorobutadiene *(in Annex A)*	HCBD
25	Pentachlorophenol	[Pentachloranisole (PCA)]
26	Polychlorinated naphthalenes	[PCN (congeners to be decided)]
	POPs listed at COP-8 (2017)	
27	Decabromodiphenyl ether (DecaBDE)	PBDE-209
28	Short-chain chlorinated paraffins	[SCCP]

(continued)

Table 2 (continued)

	Initial POPs (2001)	Recommended analytes for human milk
(24)	*Hexachlorobutadiene (in Annex C)*	*HCBD*
	POPs listed at COP-9 (2019)	
29	Dicofol	[Dicofol]
30	Perfluorooctanoic acid	PFOA
	Voluntary (POPs proposed for listing as of 2019)	
31	Medium-chain chlorinated paraffins	[MCCP]
32	Perfluorohexane sulfonic acid *)	PFHxS
	**) listed in 2022*	*[POP]: to be decided.*

of the pooled (composite) sample, sufficient sample material was available to apply different methods for determination of all 30 POPs listed until 2019 and the two POPs proposed for listing (as of 2019). Consequently, results for this complete set of 32 POPs and related analytes of interest for the Convention are available for this core matrix for the 2016–2019 period.

2.2 Assurance of the Reliability and Comparability of Results over a Long Period (2000–2019) for the Wide Range of Analytes of Interest

The guidance document on the GMP for POPs addresses numerous factors that might affect the measured concentrations of POPs in environmental samples. Sampling design is considered to be an important factor. For most POPs, the precision of chemical analysis is generally believed to constitute only a minor part of the total variance in monitoring time-series of environmental data where sample variation is expected to be large, much larger compared to laboratory precision. This is especially true when the same accredited laboratory is used throughout the whole series (as in this case). In contrast, if different laboratories carry out the analysis from year to year, it could seriously decrease or challenge the possibility to evaluate time-series of POPs. This is also true if the same laboratory changes its methodology (UNEP 2007; UNEP 2015; UNEP 2019).

To minimize possible sources of variation for time trend analysis of POPs for the effectiveness evaluation of the Stockholm Convention, the concept of the WHO/UNEP-coordinated exposure studies builds on two factors:

1. Minimization of possible sources of variation from the sampling design (see the above listed basic elements of the concept [i] for collection of a large number of individual samples fulfilling protocol criteria and [ii] preparation of pooled samples considered to be representative for a country or subpopulation at the time of sampling)

2. Minimization of the variation from chemical analysis (see basic step [iii] mentioned above regarding the performance of the analyses of the pooled samples at the Reference Laboratories)

The determination of small changes in levels of POPs is necessary and requires that variability and uncertainty in the sampling process be minimized as far as possible, while maintaining an adequate number of qualified donors. Therefore, the revised WHO protocol guidelines for the 2004–2007 study and subsequent rounds called for the recruitment of 50 individual donors per pooled sample in countries with up to 50 million population. It is recognized that some flexibility is necessary for countries with smaller populations and/or low birth rates. In some cases, reducing the number of donors was unavoidable. On the other hand, huge countries with populations well over 50 million are encouraged to prepare a second pooled sample (or more) if feasible.

The Reference Laboratory for chlorinated and brominated POPs in the period 2000–2019 was the CVUA Freiburg, Germany, and for perfluoroalkyl substances in the period 2009–2019 was at Örebro University, Sweden. Accuracy depends on systematic errors and random components. "Trueness" (closeness of the agreement between the expectation of a test result or a measurement result and a true value) and "Precision" (closeness of the agreement between independent test/measurement results obtained under stipulated conditions) are used to describe accuracy and are therefore important criteria for assessment of reliability of analytical methods. Three articles in Part II of this compendium demonstrate the application of a comprehensive long-term analytical quality control to achieve a high degree of reliability for chlorinated and brominated POPs and to estimate trueness and long-term precision (including as key elements, e.g. procedural blanks, use of fortified material, numerous quality control samples as an in-house reference material, duplicate analyses, and successful participation in proficiency tests) (Malisch and Schächtele 2023; Hardebusch et al. 2023; Schächtele et al. 2023a). The analytical methods for perfluoroalkyl substances (PFAS) were published separately (Sadia et al. 2020; Fiedler and Sadia 2021; Fiedler et al. 2022).

2.3 Cost-Effectiveness

In all WHO- and UNEP-coordinated exposure studies of human milk, only pooled samples were used for analysis. By analysis of one or a few representative pooled human milk samples for a country, an estimate of the average human body burden can be obtained, which is the result of long-term exposure in the respective different countries. The analysis of one or a few pooled human milk samples considered to be representative is far less expensive than the analysis of a high number of individual samples, particularly for PCDD and PCDF or chlorinated paraffins. Thus, this approach is very cost-effective.

2.4 Capacity Building and Global Human Milk Bank

Since 2005, the WHO guidelines for organization, sampling, and analysis specified the collection of 50 individual samples of 50 ml each. For the analysis of analytically simple POPs, such as organochlorine pesticide POPs and marker PCB, a 25 ml aliquot of each individual sample was taken and sent to a qualified laboratory chosen by the National Coordinator, usually in the country. For the pooled sample, the remaining 25 ml from each of the 50 individual samples were used to make one pooled sample and shipped frozen to CVUA Freiburg as the Reference Laboratory for chlorinated and brominated POPs for the 2000–2019 surveys. Of this pooled sample, an aliquot was sent to the PFAS Reference Laboratory at Örebro University.

The option to have individual samples analysed for the initial organochlorine pesticide POPs and marker PCB in a competent national laboratory and the analysis of pooled samples by the Reference Laboratory, is a contribution to capacity building, particularly in developing countries. The comparison of the mean of the individual samples for these analytes with the result of the Reference Laboratory serves as an external check, as the average of the results of the individual samples should be the same as the result of the pooled sample, which is prepared from equal aliquots of the individual samples. Analysis of individual samples for the analytically simple POPs also provides information on the range of levels and geographic distribution, especially when a high value is encountered.

After analysis, any remaining pooled sample was stored at CVUA Freiburg in the Global Human Milk Bank at −20 °C. This bank is used when new POPs are added to the Stockholm Convention to allow for a retrospective analysis.

2.5 Conclusion

Table 3 provides an overview on the number of countries participating in all seven rounds of WHO- and/or UNEP-coordinated exposure studies performed between 1987 and 2019, including the 82 different countries participating between 2000 and 2019, and the respective coverage of POPs, including the determination of all 30 listed POPs and two POPs recommended for listing analysed in the 2016–2019 round. All these studies were performed based on collection of individual samples from mothers according to sampling protocol criteria, the preparation of pooled samples considered to be representative for a country or subpopulation, and analysis in Reference Laboratories.

As a conclusion, the WHO/UNEP surveys are the largest global surveys on human tissues with a harmonized protocol spanning the longest time period and carried out in a uniform format. Thus, these studies are an efficient and effective tool to obtain reliable comprehensive data sets for this GMP core matrix and a key contributor to the Stockholm Convention.

Table 3 Number of countries and coverage of POPs in seven rounds of WHO- and/or UNEP-coordinated exposure studies performed between 1987 and 2019

Coordinator	Round	Period	N (countries)	Coverage of POPs
WHO	1	1987–1988	19	PCB, PCDD/PCDF
WHO	2	1992–1993	19	PCB, PCDD/PCDF
WHO	3	2000–2003	26	21 POPs
Joint WHO/UNEP	4	2005–2007	13	21 POPs
Joint WHO/UNEP	5	2008–2012	45	22 POPs
UNEP	6	2014–2015	17	22 POPs
UNEP	7	2016–2019	43	30 listed +2 proposed POPs

3 Regional Group Differentiation

Results presented in this compendium are shown for United Nations Regional Groups. The concentrations of individual POPs were compared between and within regions, however, they are not intended for ranking of individual countries. For some POPs, a wide range of concentrations with up to three orders of magnitude between lower and upper concentrations was found, even for countries in the same UN Regional Group. Some countries had levels within the usual range for most POPs, but high concentrations for a certain POP. Findings of concentrations in the upper third of the frequency distribution may motivate targeted follow-up studies rather than if the observed level of a POP is found in the lower third of frequency distribution. However, the levels of concentrations have also to be seen in context with the sampling period and POPs use patterns/history in each country.

Comprehensive articles in Part III and Part IV of this review book present the complex discussion for the 32 POPs of interest in detail. Further, two selected examples (PCB and DDT) below illustrate some important regional aspects. For the convenience of readers, the supplementary information for this article (appendix, section "Regional Differentiation") contains short summaries of regional aspects also for other selected POPs: (i) Toxic equivalents [TEQ] of PCDD/PCDF and dioxin-like PCB [WHO_{2005}-TEQ]); (ii) beta-HCH; (iii) HCB; (iv) dieldrin; (v) PBDE; (vi) PFOS; and (vii) chlorinated paraffins. For all other POPs and the detailed discussion refer to the articles mentioned above.

3.1 PCB

The range of concentration levels detected for PCB (expressed as the sum of 6 indicator PCB [ΣPCB_6]) in 232 pooled human milk samples from 82 countries collected between 2000 and 2019 varied approximately between 1 and 1000 ng/g lipid. The highest concentrations were found in the Eastern European Group (median of 121 ng/g lipid and maximum of 1009 ng/g lipid), followed by the group of

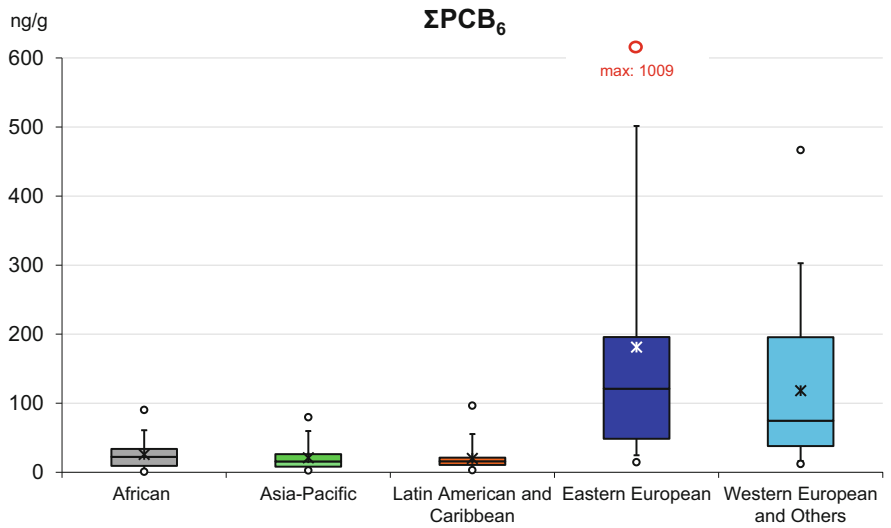

Fig. 1 Range of concentrations of the sum of 6 Indicator PCB (ΣPCB6) among UN Regional Groups (ng/g lipid, upper bound; $N = 232$ pooled samples from 82 countries) [Box plot; minimum and maximum: as circles; fifth and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

Western European and Other States (median 75 ng/g lipid, maximum 467 ng/g lipid). In all other groups, considerably lower PCB levels were found (median between 15 and 22 ng/g, maximum lower than 100 ng/g lipid) (Fig. 1). Note that Australia, Israel, New Zealand, and the USA are included as "Others" in the Western European and Others Group; in these countries, lower PCB concentrations were found comparable to non-European countries. Overall, observed PCB concentrations were considerably higher in Europe than in the other geographic regions.

Figure 2 illustrates the regional differentiation (indicating the five UN Regional Groups by different colours) with temporal tendencies over the five rounds. Between 2000 and 2003, the majority of participants were European countries. Later, WHO and UNEP encouraged countries of other groups to participate through special programmes. As shown above, European countries had by far the highest ΣPCB_6 concentrations. This is supported by the results of the 2000–2003 round, when only Eastern and Western countries were found in the upper third and middle part of the frequency distribution. The 2004–2007 round also showed considerably higher non-dioxin-like PCB concentrations in European countries. In these rounds, the highest ΣPCB_6 concentrations were found in samples from the Czech and the Slovak Republics. In one of the three pooled samples submitted by the Czech Republic in 2001, the highest concentration of 1009 ng/g was found. The median of the three submitted samples was 502 ng/g. However, the PCB concentrations in samples of these two countries decreased considerably to 109 ng/g in the Czech Republic and 78 ng/g in the Slovak Republic in the samples from 2019. For a detailed discussion, see Part III of this compendium (Malisch et al. 2023b).

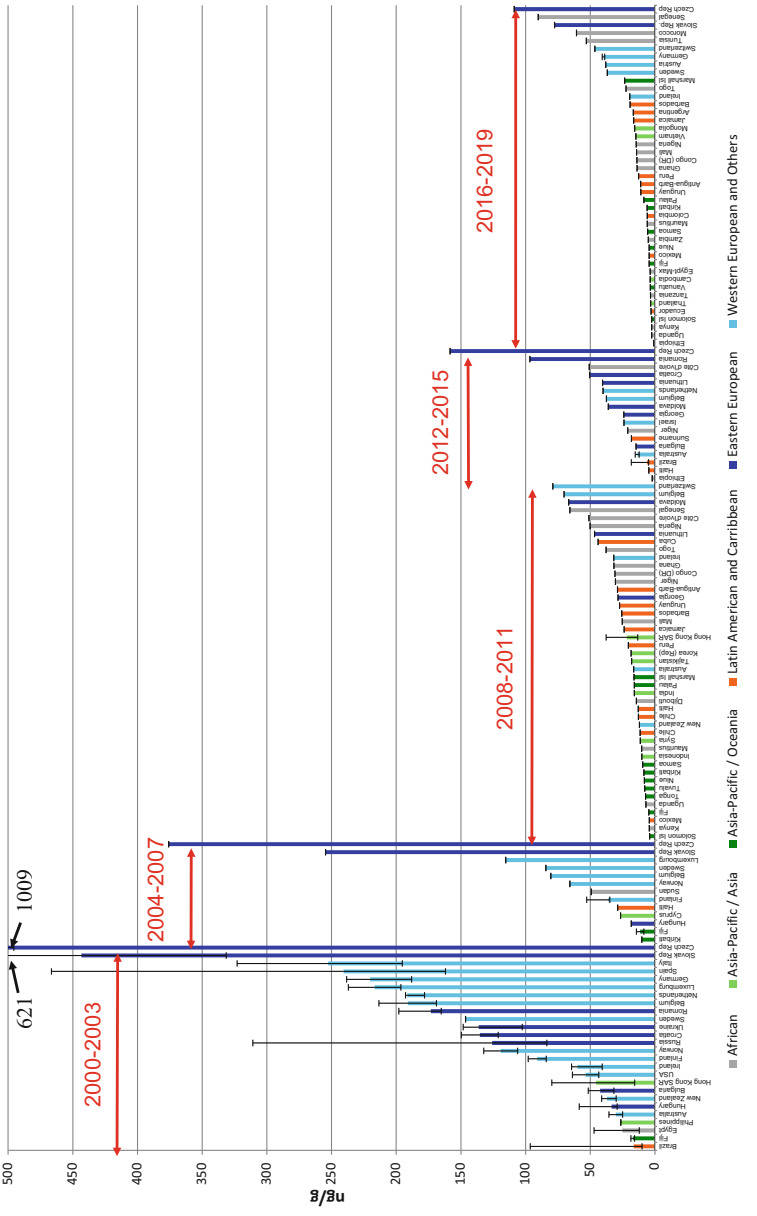

Fig. 2 Results of the 2000–2019 surveys for the sum of 6 Indicator PCB (ΣPCB_6) in 232 pooled human milk samples from 82 countries of the five UN Regional Groups (with split of the Asia-Pacific Group into two subgroups Asia and Pacific Islands) indicated by different colours and classified into five periods of sample submission (ng ΣPCB_6/g lipid). Whiskers indicate the range of concentrations found in the pooled samples around the median concentration, if two or more samples were submitted by a country in a certain period

3.2 DDT

The highest median levels of DDT (expressed as "DDT complex": sum of all detected analytes, calculated as DDT [Malisch et al. 2023c]) were found in the African Group (517 μg DDT complex/kg lipid), followed by the Eastern European Group (449 μg DDT complex/kg lipid), the lowest median levels in the Western European and Others Group (147 μg DDT complex/kg lipid) (Fig. 3). However, large differences were seen between countries even in the same region. In the African Group, the range of DDT complex concentrations stretched over three orders of magnitude with a minimum of 17 μg/kg lipid found in 2019 in one country and a maximum of 23,500 μg/kg lipid found in 2012 in another country. The DDT complex concentrations in the Asia-Pacific Group covered a range between 189 and 8500 μg/kg lipid.

In all UN Regional Groups, the median of the DDT complex concentrations was higher in the 2000–2003 period than in the 2016–2019 period. However, in the three rounds in-between, a considerable variation within the regions was observed; caused by the variation of countries participating in a certain UN Regional Group in a certain period. In this way, the elevated concentrations in the 2008–2011 period were found in Tajikistan (8500 μg DDT complex/kg lipid) and on the Solomon Islands (4760 μg DDT complex/kg lipid), and in the 2012–2015 period in Ethiopia (23,500 μg DDT complex/kg lipid). However, the 2019 samples from the Solomon Islands and Ethiopia showed considerable downward trends with 1390 and 7100 μg DDT complex/kg lipid, respectively (Fig. 4). For a detailed discussion, see Part III of this compendium (Malisch et al. 2023c).

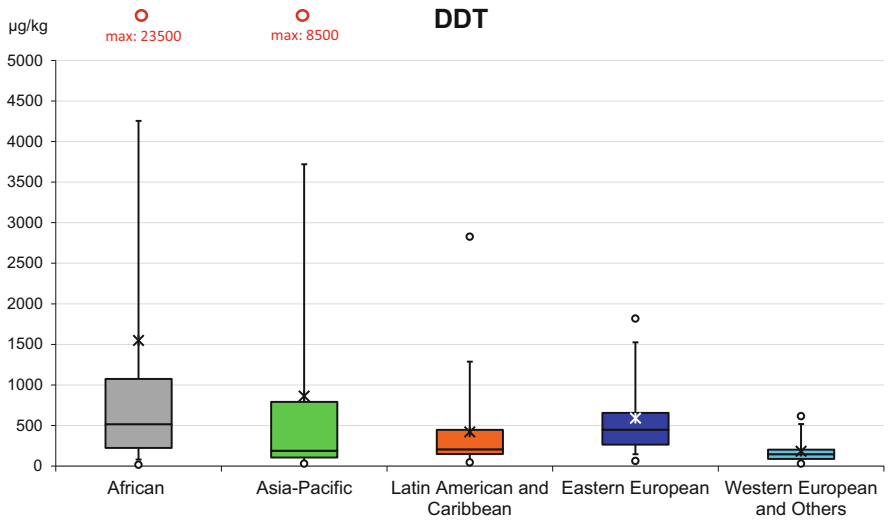

Fig. 3 Range of concentrations of DDT (expressed as μg DDT complex/kg lipid) in human milk among UN Regional Groups ($N = 134$ country results [using the median, if results for two or more pooled samples are available in a certain period] from 82 countries, comprising the five periods from 2000–2019) [Box plot; minimum and maximum: as circles; fifth and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

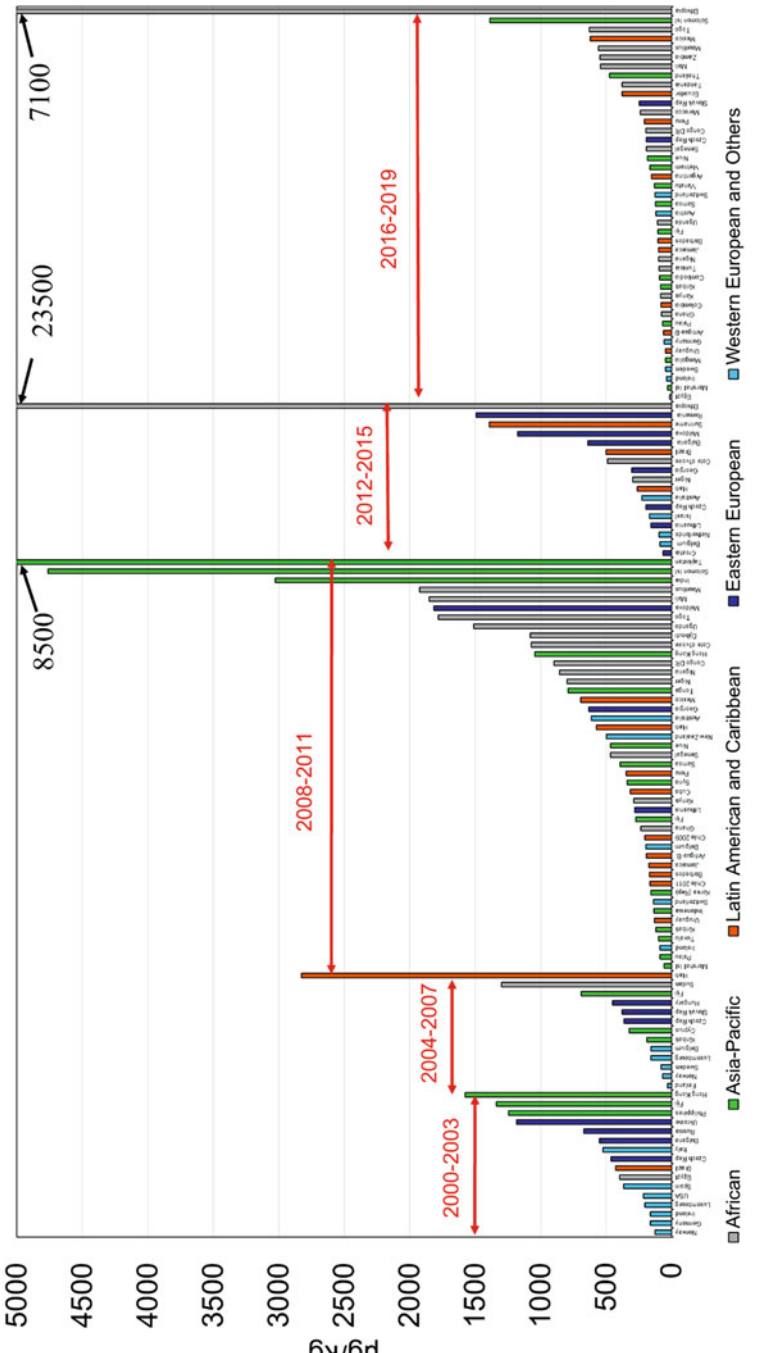

Fig. 4 Concentrations of DDT (expressed as µg DDT complex/kg lipid) in human milk from 82 countries of the five UN Regional Groups for five periods from 2000 to 2019 ($N = 134$ country results [using the median, if results for two or more pooled samples are available in a certain period])

4 Assessment of Time Trends

The estimation of time trends based on data from different countries reported in the literature is difficult, as different sampling concepts were applied and the comparability of the analytical results is not always clear (Fürst 2023). However, for the long-term, the aim of the WHO/UNEP-coordinated exposure studies is to support the Convention's implementation by providing data to the effectiveness evaluation as required under Article 16. Therefore, as explained above in subsection 2.2, possible sources of variation for time trend analysis were minimized by the concept building on the two factors (sampling design; performance of the analyses of the pooled samples at the Reference Laboratories).

For the evaluation of time trends, participation in different sampling periods is necessary. Countries were encouraged to participate repeatedly. The first sampling period is conducted to determine baseline concentrations for POPs in randomly selected individual samples of human milk and pooled samples made from them. A second sampling period should be conducted with a similarly selected cohort, e.g. four or five years later (or other time period deemed appropriate). Future samplings should be undertaken at regular intervals and the monitoring of human milk for POPs should be considered a long-term activity.

To provide reliable and comparable monitoring information for the Parties to the Stockholm Convention, the GMP guidance document proposed a quantitative objective for temporal trend studies: These studies should be able to detect a 50% decrease in the levels of POPs within a 10-year period (UNEP 2015; UNEP 2019). However, in distinction from this goal for abilities of temporal trend studies to detect changes over time, there is no stipulation of a quantitative goal for the rate of reduction/decrease in POPs levels by the Convention. The Convention's objectives are either to eliminate or to restrict production, use, and releases, depending on the annex where a chemical is listed, but the rate of decline is nowhere specified.

A period of at least 10–15 years is estimated to be necessary to detect significant temporal changes of moderate size for most POPs. For example, a change of 7% per year would be necessary for a 50% decrease over 10 years. Furthermore, at least 4 to 5 years of monitoring would be necessary to give reliable estimates taking into account random within- and between-year variation and other components of variance (UNEP 2019). In addition, the rate of decrease will vary among POPs. Moreover, the reduction rates depend on the concentration range (levels above or in the range of background contamination), see subsection 4.1 "DDT" in the following.

The estimation of time trends based on comparison of median or mean concentrations in UN Regional Groups in the five studies by classification into equal four-year periods between 2000 and 2019 provides a first orientation. However, the variation of countries participating in a certain UN Regional Group in a certain period can influence the median or mean concentrations. In particular, if the number of countries differs considerably between periods or if quite high concentrations are found in a sample submitted by a country with single participation in a certain period, the resulting median or mean can be distorted. Thus, it is more precise to only use results from countries with repeated participation in these studies:

This allows drawing conclusions on temporal trends, which are not potentially influenced by these possible factors.

In the following, short summaries of three examples of the "initial POPs" (DDT; PCB; Toxic equivalents of PCDD/PCDF and dioxin-like PCB [WHO_{2005}-TEQ]) and of one emerging POP (chlorinated paraffins [CP]) illustrate these important aspects for conclusions on time trends. For the convenience of readers, more examples are given in the supplementary information (appendix, section "Assessments of Time Trends"): (1) beta-HCH; (2) HCB; (3) PBDE; (4) PFOS. For other POPs and the full view, comprehensive articles appear in Part III and Part IV of this compendium.

4.1 DDT

As general estimation of time trends, the **median** of the DDT complex concentrations of all country results ($N = 134$) shows a decrease of 72% from the 2000–2003 period (median for 16 countries: 445 μg/kg lipid) to the 2016–2019 period (median for 43 countries: 125 μg/kg lipid). The downward trend between these two periods was found in all regions, but with considerable differences among regions and a great variation among the three rounds in-between, as illustrated by Fig. 5. In all groups, the median of the DDT complex concentrations was higher in

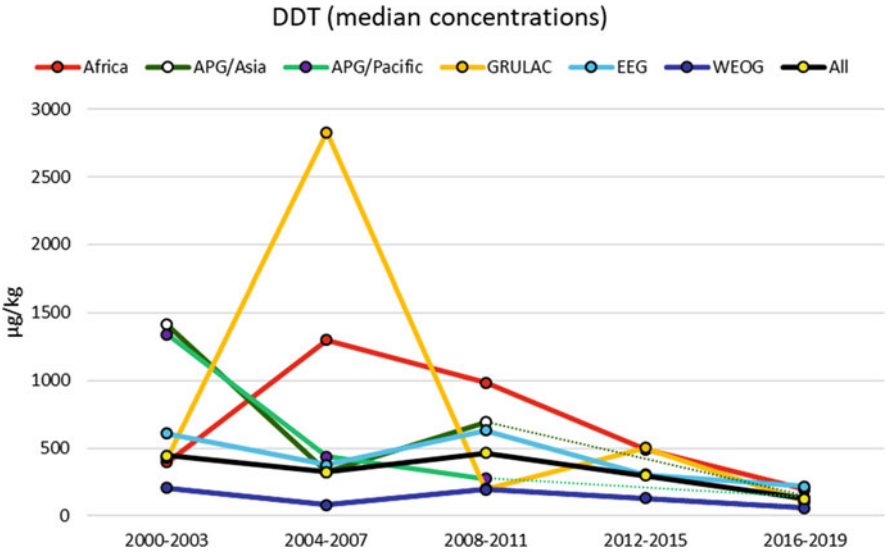

Fig. 5 Time trends for median concentrations of DDT (expressed as μg DDT complex/kg lipid) in human milk in the five UN Regional Groups (with split of the Asia-Pacific Group [APG] into two subgroups Asia and Pacific Islands; GRULAC = Group of Latin American and Caribbean Countries; EEG = Eastern European Group; WEOG = Western European and Others Group) in five periods between 2000 and 2019 based on all country results (using the median, if results for two or more pooled samples are available in a certain period) from 82 countries

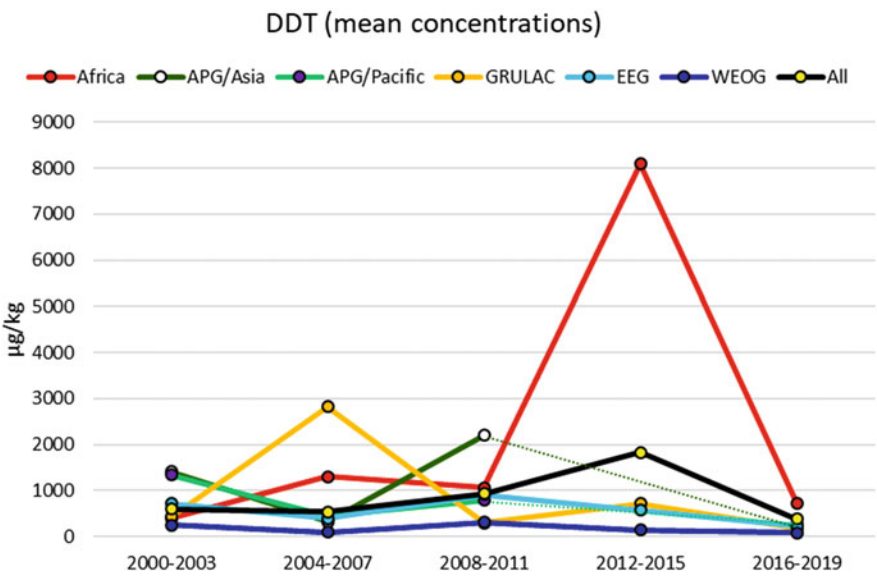

Fig. 6 Time trends for mean concentrations of DDT (expressed as µg DDT complex/kg lipid) in human milk in the five UN Regional Groups (with split of the Asia-Pacific Group [APG] into two subgroups Asia and Pacific Islands; GRULAC = Group of Latin American and Caribbean Countries; EEG = Eastern European Group; WEOG = Western European and Others Group) in five periods between 2000 and 2019 based on all ($N = 134$) country results (using the median, if results for two or more pooled samples are available in a certain period) from 82 countries

the 2000–2003 period than in the 2016–2019 period. However, in the three rounds in-between, a considerable variation within UN Regional Groups was observed; obviously with a substantial maximum in Latin American and Caribbean countries in the 2004–2007 period and elevated levels also in Africa at that time period. If calculated as the **mean** of all country results, the DDT complex concentration was higher in the 2000–2003 period than in the 2016–2019 period in all regions except Africa. Again, in the three rounds in-between, a considerable variation was observed, with a substantial maximum in Africa in the 2012–2015 period. This shows that the mean is misleading, if substantial maxima occur (Fig. 6).

As a result, the median, mean, and ranges of DDT complex concentrations found in the UN Regional Groups in five periods over these 20 years cannot be used directly to derive continuous time trends. The main problem is the variation of countries and the low number of countries participating from a certain UN Regional Group in a certain period, in particular, if a country with a very high concentration participated in a single period: This could have a considerable effect on median or mean concentrations for this UN Regional Group in that period. The substantial maximum in Latin American and Caribbean countries in the 2004–2007 period was caused by high DDT concentrations found in Haiti in 2005 (2830 µg/kg lipid) as only country of this UN Regional Group in this period; however, in Haiti, these concentrations decreased considerably in the following years (in 2011: 574 µg/kg

lipid, in 2015: 263 µg/kg lipid). The maximum of the mean values in African countries in the 2012–2015 period was caused by the high DDT concentration in human milk from Ethiopia in 2012 (23,500 µg/kg lipid; participating as one of three African countries in this period). However, also in Ethiopia, the DDT concentrations decreased considerably until 2019 (7100 µg/kg lipid). For more information and discussion of the findings of DDT in UN Regional Groups, see (Malisch et al. 2023c).

The assessment of time trends based only on results of countries with repeated participation allows more certainty in drawing the conclusions, and therefore is advisable for the evaluation of changes over time for the effectiveness evaluation for the purpose of Article 16. Figure 7 illustrates the temporal tendencies observed in 44 countries of the five UN Regional Groups that participated in the survey two or more times. In nearly all countries, decreasing tendencies are observed, with a median of 59% decrease over the years between the first and the last participation.

The reduction rates in countries should be seen in context and discussed within the total concentration range: A differentiation among levels above or within the range of the background contamination seems to be necessary. If high levels are found, sources might be detected which could be eliminated, as e.g. described for Ethiopia (Gebremichael et al. 2013). However, at low/background levels, other factors, e.g. secondary contamination of feeds and food via long-range atmospheric transport and subsequent bioaccumulation, cannot be influenced locally.

It should also be noted that a limited number of data points of concentrations over the period of 2000–2019 are available from the individual countries which prevents the determination of statistically significant individual temporal trends in most cases. Nevertheless, existing data do indicate decreasing or increasing tendencies in POP concentrations. Furthermore, pooling of/combining data collected in regions allows deriving statistically significant time trends in the UN Regional Groups and overall globally.

For the 44 countries with repeated participation, the overall decrease of POP concentrations per 10 years was calculated by the non-parametric linear Theil–Sen trend estimator (Sen 1968; Theil 1992). Secondly, a method for deriving the regional group trend as a median of trends in countries within the group was used ("median method") (for methods of statistical data treatment, see Malisch et al. 2023d). A statistically significant overall decrease within a 10-year period between 50% and 80% was achieved for DDT levels in the African Group, the Asian-Pacific Group and the Group of Latin American and Caribbean Countries, and at a global level. Lower decrease rates were observed in the Eastern European Group and the Western European and Others Group, which had banned DDT much earlier. As the median of the five UN Regional Groups, a decrease per 10 years by 58% was found for DDT (Table 4). For more details, see (Malisch et al. 2023e).

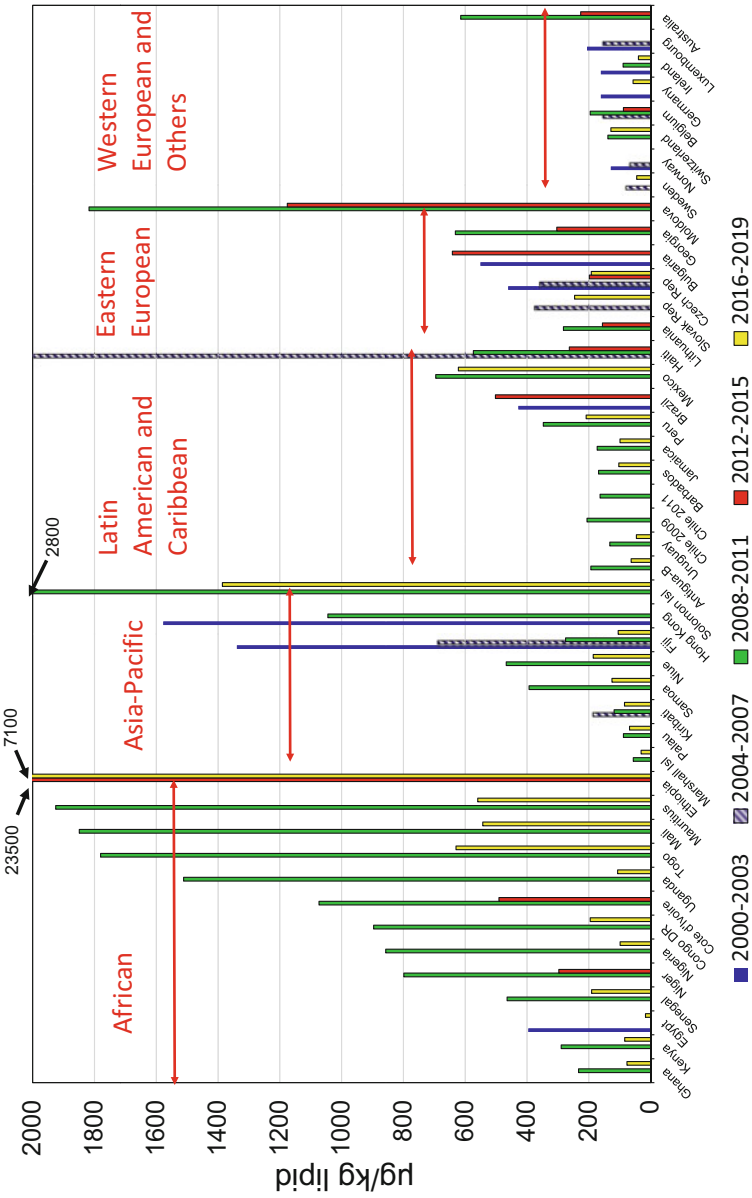

Fig. 7 Overview of the development of DDT concentrations in human milk (μg DDT complex/kg lipid) over time between 2000 and 2019 for 44 countries of the five UN Regional Groups that participated two or more times

Table 4 Overall decrease (%) of DDT concentrations (calculated as DDT complex) in human milk in the five UN Regional Groups and worldwide (computed using all samples submitted by countries with repeated participation)

UN Regional Groups	*N* of countries	Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	
African	13	74.3	79.1	<0.001
Asia-Pacific	8	80.2	66.5	<0.001
Latin American and Caribbean	9	49.5	55.0	0.017
Eastern European	6	38.5	45.5	0.017
Western European and others	8	39.1	48.6	<0.001
Global	44	58.3	49.5	<0.001

4.2 PCB

As shown above (subsection 3.1), in comparison to other geographic regions, European countries had by far the highest ΣPCB_6 concentrations (Indicator PCB) in the 2000–2019 period. This period can be expanded by the inclusion of results for non-dioxin-like PCB of the first (1987–1988) and second (1992–1993) rounds of WHO-coordinated exposure studies (WHO 1989, 1996), to cover in total about three decades. For the assessment of temporal trends based only on countries with repeated participation, data from 25 European countries are available, of which 15 countries participated already in the 1987–1988 and/or the 1992–1993 round (Fig. 8).

At the end of the 1980s and early 1990s, ΣPCB_6 concentrations in European countries were in a range up to 800 ng/g lipid and decreased until 2019 to concentrations mostly below 100 ng/g lipid. After detection of PCB in environmental samples in 1966 and measures taken since the 1970s, significant reductions were already achieved in the 1990s. Therefore, a decrease of ΣPCB_6 concentrations in human milk by up to 95% was achieved since the end of the 1980s and resulting in low background levels observed in 2019.

Also, in most countries from the other UN Regional Groups, a decrease between different periods was observed, but these decreases began at considerably lower concentrations (below about 100 ng/g lipid). Similarly to 15 European countries, two Asian countries (Vietnam, Thailand) were monitored over three decades (with first participation already in the 1987–1988 POPs exposure study), whereas the studies in some African, Latin American and Caribbean countries comprise at best two decades (with first participation in the 2000–2003 or 2004–2007 study) but rather a decade—starting from the 2008–2011 survey round (Fig. 9).

In the majority of UN Regional Groups and at a global level, a decrease between 50% and 70% within a 10-year period was achieved for the levels of ΣPCB_6. The Latin American and Caribbean countries had lower ΣPCB_6 concentrations in

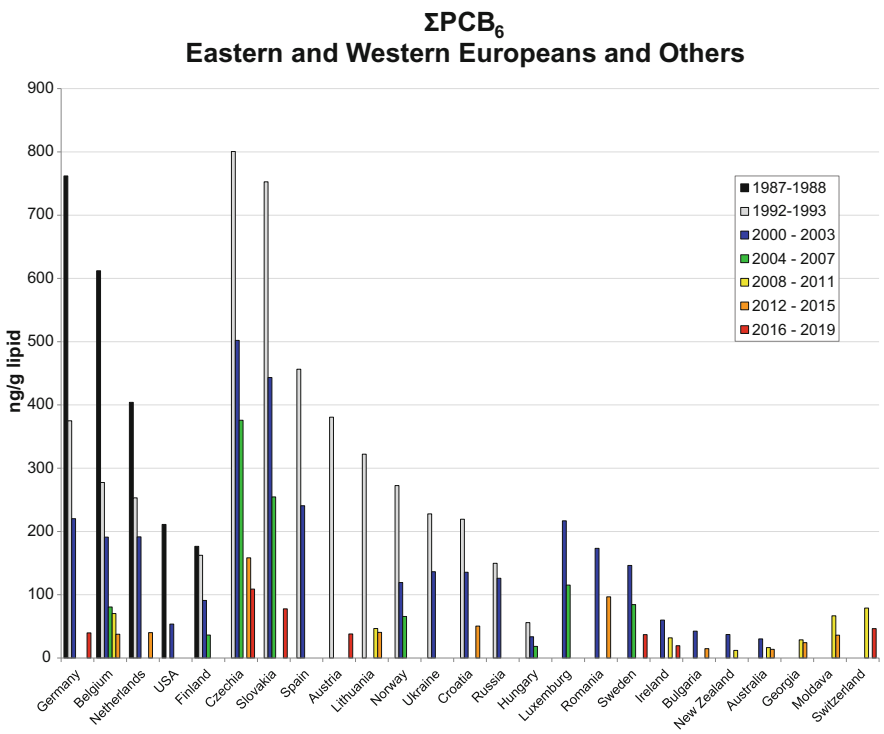

Fig. 8 Overview of the development of Indicator PCB concentrations (ng ΣPCB_6/g lipid; country results as median of multiple sample results, if two or more pooled samples were submitted in a certain period) over time between 1987 and 2019 for 25 countries of the Eastern European and Western European and Others Regional Groups that participated two or more times

comparable periods, obviously resulting in lower decrease rates. This is an indication that the decrease might be faster in regions with higher concentration, compared to a slower decrease in less polluted regions. All trends were statistically significant (p-value <0.001) (Table 5).

For details of the first general estimation of temporal trends from all 82 countries participating between 2000 and 2019, see (Malisch et al. 2023b); for assessment of time trends only of 57 countries with repeated participation in WHO/UNEP-coordinated exposure studies performed between 1987 and 2019, see (Malisch et al. 2023d).

4.3 Toxic Equivalents of PCDD and PCDF (WHO-PCDD/PCDF-TEQ [2005])

As for non-dioxin-like PCB, also for PCDD and PCDF data are available from the first two rounds of human exposure studies coordinated by WHO in the mid-1980s

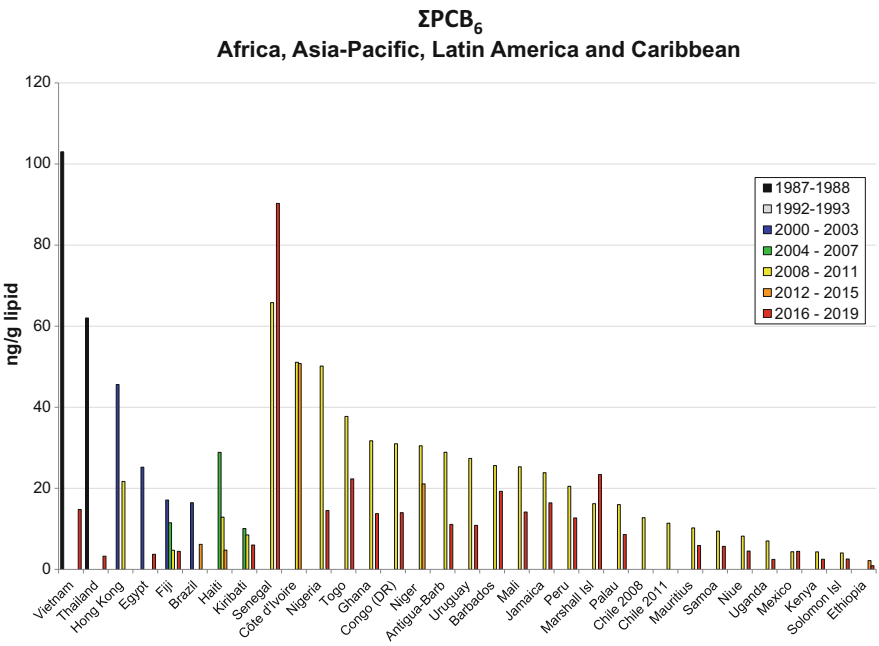

Fig. 9 Overview of the development of Indicator PCB concentrations (ng ΣPCB_6/g lipid; country results as median of multiple sample results, if two or more pooled samples were submitted in a certain period) over time between 1987 and 2019 for 32 countries of the African Group, the Asia-Pacific Group and the Group of Latin American and Caribbean Countries that participated two or more times

Table 5 Overall decrease (%) of Indicator PCB (ΣPCB_6) concentrations in the five UN Regional Groups and globally (computed using all samples submitted by countries with repeated participation)

UN Regional Group	*N* of countries	Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	
Africa	13	49.5	56.7	<0.001
Asia-Pacific	10	64.8	51.2	<0.001
Latin American and Caribbean	9	34.0	45.1	<0.001
Eastern Europe	11	53.3	55.8	<0.001
Western Europe and others	14	62.8	61.9	<0.001
Global	57	70.5	53.3	<0.001

and 1990s to determine the concentrations of PCB, PCDD, and PCDF in human milk. As dioxin-like PCB were of interest starting from the second WHO round (1992–1993), time trends for total TEQ (WHO_{2005}-TEQ) can be derived only beginning with that period. Therefore, the time trends for WHO-PCDD/PCDF-TEQ (calculated using the toxic equivalency factors [TEF]) proposed in 2005 [WHO_{2005}-TEF] [van den Berg et al. 2006]; for calculation and use of the terms for sum parameters for toxic equivalents including "WHO-PCDD/PCDF-TEQ [2005]", see Malisch et al. 2023b) are summarized, which can be derived beginning with the 1987–1988 period covering again about three decades. For the assessment of temporal trends of PCDD and PCDF based only on countries with repeated participation, data from 19 countries (15 European and New Zealand, Thailand, USA, and Vietnam) are available, which participated already in the 1987–1988 and/or the 1992–1993 round (Fig. 10). In the Netherlands, Belgium, and Germany with highest WHO-PCDD/PCDF-TEQ concentrations in the 1987–1998 period, a decrease of about 90% was achieved until the 2012–2015 or 2016–2019 period.

In the UN Regional Group with Western European and other countries with relatively high WHO-PCDD/PCDF-TEQ concentrations in human milk in the 1987–1988 and 1992–1993 rounds, a decrease of 51% over 10 years was achieved. Most Eastern European countries participated for the first time in the 1992–1993 period with on average lower WHO-PCDD/PCDF-TEQ concentrations in human

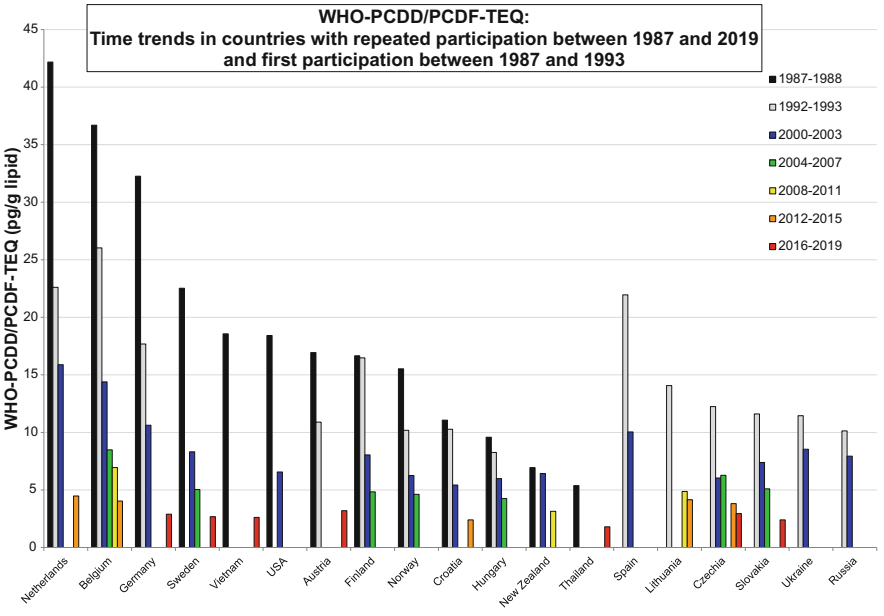

Fig. 10 Overview of the development of WHO-PCDD/PCDF-TEQ (2005) concentrations (pg/g lipid; country results as median of multiple sample results, if two or more pooled samples were submitted in a certain period) over time for countries with repeated participation in WHO/UNEP-coordinated exposure studies between 1987 and 2019 and first participation in the first or second round

Table 6 Overall decrease (%) of WHO-PCDD/PCDF-TEQ concentrations in the 5 UN Regional Groups and globally (computed using all samples submitted by countries with repeated participation)

UN Regional Group	*N* of countries	Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	
Africa	13	65.5	10.2	<0.001
Asia-Pacific	10	45.9	20.5	<0.001
Latin American and Caribbean	9	14.7	33.7	<0.001
Eastern Europe	11	35.3	37.5	<0.001
Western Europe and others	14	50.9	47.9	<0.001
Global	57	47.9	45.9	<0.001

milk than in Western European countries; for this group the decrease within a 10-year-period was 35%. Large differences between the decrease calculated by the Theil–Sen method or the median method indicate a considerable variation between countries in that region. Lower decrease rates were observed in some countries, but this has to be seen in context with the quite low initial levels in these countries. Therefore, these regional trends have also to be seen in context with the variation among participating countries. As median of the five UN Regional Groups, a decrease per 10 years by 48% was found for WHO-PCDD/PCDF-TEQ (Table 6). For more details and for the assessment of temporal trends of total TEQ (WHO_{2005}-TEQ), see (Malisch et al. 2023d).

4.4 Chlorinated Paraffins

Average CP levels have increased between two time spans (2009–2014 vs 2015–2019) in four out of the five UN Regional Groups: in the African Group (AFR), the Asia-Pacific Group (ASPAC), the Eastern European Group (EEG), and the Group of Latin America and Caribbean Countries (GRULAC) (Fig. 11). On the other hand, average CP levels in the Western European and Others Group (WEOG) seem to have decreased in the latter time period, with a comparatively close grouping of the results. As only two data points per country were available, it is not possible to derive statistically significant time trends for individual countries but statistically not significant temporal tendencies (Krätschmer et al. 2023).

Temporal trends over a ten-year period of the CP levels in the five UN regional groups were assessed by the Theil–Sen method. No significant changes in CP levels were observed in the African and GRULAC groups as a whole, while a decrease by 63% was calculated in the WEOG group over 10 years ($p = 0.001$). In contrast, in the Asia-Pacific and Eastern European regional groups an increase of CP over a

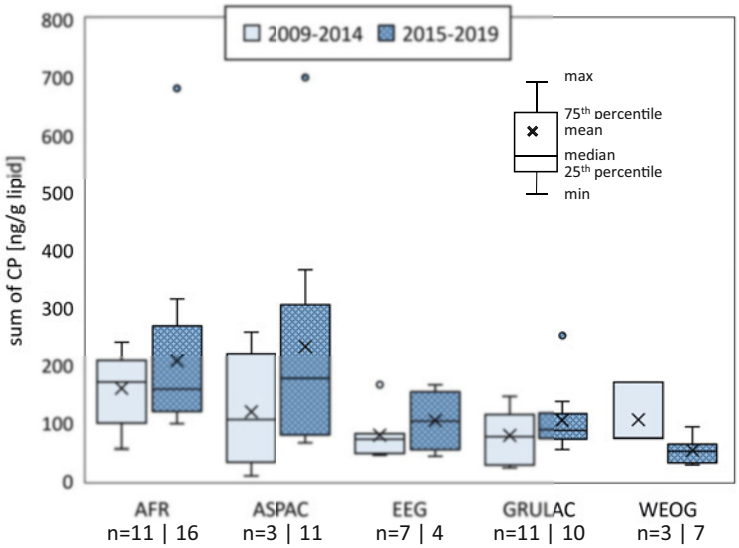

Fig. 11 Range of sum of CP determined in pooled human milk samples from the 2009–2019 period of WHO/UNEP-coordinated human milk studies. 27 of the samples collected before 2015 were only analysed for their total CP content, for all other samples, SCCP and MCCP levels were reported. AFR: Africa, ASPAC: Asia-Pacific Group, EEG: Eastern European Group, GRULAC: Group of Latin American and Caribbean Countries, WEOG: Western European and Others Group (including Australia)

Table 7 Overall decrease (%) of total CP concentrations in the 5 UN Regional Groups and worldwide (computed using all individual samples). Negative decreases are to be read as increase. (n = number of samples)

UN Regional Groups	n	Overall decrease per 10 years [%]	p-value
African	27	−6.3	0.663
Asia-Pacific	15	−179	0.009
Latin American and Caribbean	21	9.5	0.737
Eastern European	11	−197	0.007
Western European and others	10	63	0.001
Global	84	−29	< 0.001

decade by up to 200% was calculated ($p < 0.010$). The ten-year-trend globally, based on all 84 results of 57 countries covering the period between 2009 and 2019, indicated an increase of total CP in human milk by 30% ($p < 0.001$) (Table 7) (Krätschmer et al. 2023). This indication of increasing CP levels in the background contamination of human milk is cause for concern.

5 Relative Importance of POPs

Another important aspect is the share of the individual 30 POPs and 2 additional chemicals proposed for listing in "total POPs concentrations". Results for the complete set of these "30 plus 2" POPs of interest are available for the core matrix "human milk" for the 2016–2019 period—a unique characteristic among the core matrices under the GMP. For discussion of the relative importance ("ranking"), the following differentiation is necessary:

- The *lipophilic chlorinated and brominated POPs* are reported on lipid base. Here, **dioxin-like compounds** (PCDD, PCDF, and dioxin-like PCB contributing to toxic equivalents [TEQ]); furthermore PCN, which according to peer reviewed publications have also dioxin-like toxic toxicity, have to be determined in the **pg/g range**, whereas the **other chlorinated and brominated POPs** are usually determined in the **ng/g (=µg/kg) range**.
- The more polar *perfluoroalkyl substances (PFAS)* data are usually reported on product base (as **pg/g fresh weight**) or on volume base (**ng/L**).

5.1 Dioxin-Like Compounds

In the 44 samples submitted in the 2016–2019 period, total TEQ (WHO_{2005}-TEQ) resulting from PCDD, PCDF, and dioxin-like PCB were in the range 1.29–11.6 pg/g lipid (median: 3.90 pg/g lipid). On average, 73% resulted from PCDD and PCDF (range 44–92%; median: 2.63 pg WHO-PCDD/PCDF-TEQ/g lipid, range 1.02–9.97 pg/g lipid) and 27% from dioxin-like PCB (range 8–56%; median: 1.00 pg WHO-PCB-TEQ/g lipid, range 0.27–3.70 pg/g lipid) (Malisch et al. 2023b).

With regard to dioxin-like compounds (DLCs), at present there is no health-based guidance value (HBGV) available for the breastfed infant. Although formally these HBGVs have been set to protect human health for a lifetime exposure period, much of the underlying experimental data focus on the perinatal and/or childhood period. Therefore, it is justifiable to use these HBGVs for early life and shorter than lifetime exposures, e.g. breastfeeding. With this approach the present HBGVs for DLCs were generally exceeded one order of magnitude or more in industrialized countries over the period 2000 to 2019. If HBGVs of 1 or 0.1 pg TEQ/kg/day are used to calculate toxicological acceptable levels of DLCs in human milk, it can be estimated that such levels will not be reached before, respectively, 2030 or 2050. When the subtle adverse health effects of DLCs in the breastfed infant reported in the 1990s were compared with benefits of breastfeeding for the infant and mother, it is concluded that benefits grossly outweigh the potential adverse health. Therefore, it is concluded that the WHO has rightfully encouraged breastfeeding for the last decades (van den Berg et al. 2023).

The concentrations of polychlorinated naphthalenes (PCN) were determined in 40 pooled human milk samples from 39 countries (Tschiggfrei et al. 2023). The median concentration of the sum of 26 PCN was 55 pg/g lipid (range 27 to 170 pg/g).

Among other adverse biological effects, PCN also show dioxin-like toxicity and this was estimated by calculating the toxic equivalents in these samples using two sets of relative effect potency (REP) values: a set that has been used in earlier human exposure studies, and REPs suggested by Falandysz et al. (2014). The median PCN-TEQ concentration in human milk was 0.07 pg PCN-TEQ/g lipid (range 0.03 pg/g to 0.23 pg/g), when calculated using the human biomonitoring study REPs, and 0.03 pg PCN-TEQ/g lipid (range 0.01 pg/g to 0.10 pg/g), when calculated with other suggested REPs. The vast majority, about 90%, of this TEQ can be attributed to the PCN 66/67 congener pair. The assessment of PCN 66 and PCN 67 in order to obtain confirmed TEFs would be most important for calculations of the dioxin-like toxicity of PCN congeners, followed by PCN 69. Minor contributions to PCN-TEQ concentrations in human milk come from PCN 52/60, PCN 64/68, PCN 70, and PCN 73.

On average, the contribution of PCN-TEQ to the cumulative TEQ (including the overall sum of toxic equivalents of PCDD, PCDF, and dioxin-like PCB [WHO_{2005}-TEQ]) was between 1% and 2%, with a wider range up to 5% for the 39 countries of this study. This is about an order of magnitude lower than the contribution of dioxin-like PCB to the cumulative TEQ (median 26%). In line with the observed higher total PCN concentrations, European countries also showed considerably higher levels of PCN-TEQ than the other regions. PCN-TEQ calculated with REPs used in human biomonitoring studies add on average about 2% to the cumulative TEQ of dioxin-like contaminants in Africa, Asia-Pacific and Latin American and Caribbean countries and about 4% in European countries. The corresponding contribution of PCN-TEQ calculated with REPs suggested by Falandysz et al. (2014) would be 1% in non-European countries and 2% in European countries.

In addition to the 30 chemicals listed by the Convention and 2 chemicals proposed for listing as of 2019, in 38 pooled samples from 28 countries collected between 2001 and 2009 concentrations of polybrominated dibenzo-*p*-dioxins and -furans (PBDD/PBDF) were determined to assess their contribution to the total TEQ (WHO_{2005}-TEQ) resulting from PCDD, PCDF, and dioxin-like PCB. PBDD and PBDF provided on average about 10% to the overall TEQ calculated as sum of WHO-PCDD/PCDF-TEQ and WHO-PBDD/PBDF-TEQ, when assuming the same TEFs for brominated congeners as assigned to their chlorinated analogues. No correlations between PCDD/PCDF and PBDD/PBDF, or PBDD/PBDF and PBDEs (calculated as $\sum PBDE_6$) were found (Kotz et al. 2005; Schächtele et al. 2023b).

5.2 Non-dioxin-Like Chlorinated and Brominated POPs

The range of the non-dioxin-like chlorinated and brominated POPs found in the 2016–2019 survey is illustrated by Figs. 12 and 13. By far highest concentrations were found for DDT and for chlorinated paraffins (total CP including SCCP and MCCP). The maximum found for DDT (7100 ng DDT complex/g lipid) was a factor of 10 higher than the maximum of total CP (700 ng/g lipid for the sum of SCCP and

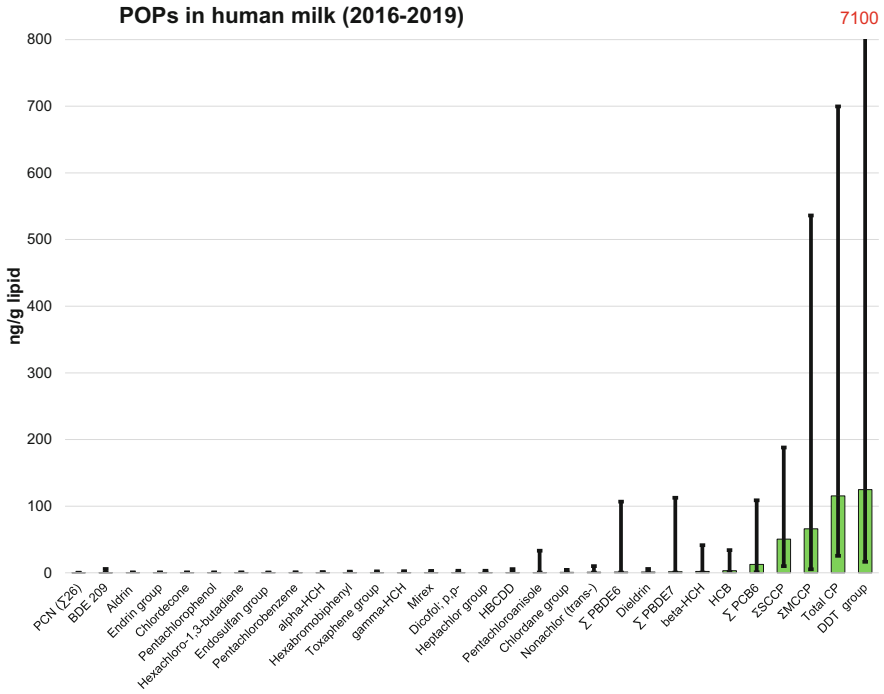

Fig. 12 Range of concentrations of lipophilic chlorinated and brominated POPs in human milk (ng/g lipid) from 43 countries in the period 2016–2019 (median with error bars indicating the minimum and maximum)

MCCP). However, the median of CP concentrations (116 ng total CP/g lipid) was comparable to the median of DDT concentrations (125 ng DDT complex/g lipid). The high CP concentrations were caused predominantly by MCCP (median 66 ng/g lipid; maximum 536 ng/g lipid), with SCCP concentrations of 51 ng/g lipid as median and 188 ng/g lipid as maximum. PCB as next following group in the ranking had on average an order of magnitude lower concentrations than the total CP concentrations (median 12.7 ng $\sum PCB_6$/g lipid, maximum 109 ng/g lipid).

Median concentrations between 1.0 ng/g lipid and 3.3 ng/g lipid were found for nonachlor, $\sum PBDE_6$ (and $\sum PBDE_7$ including BDE-209), dieldrin, beta-HCH, and HCB; maximum levels between 10 ng/g lipid and 110 ng/g lipid for pentachloroanisole, nonachlor, beta-HCH, HCB, and $\sum PBDE_6$. The concentrations of other chlorinated and brominated POPs were frequently below LOQ (0.5 ng/g lipid) or, if quantifiable, below 10 ng/g lipid. The quite low concentrations for $\sum PCN_{26}$ (median 55 pg/g lipid; range 27 to 170 pg/g) have to be assessed with regard to their dioxin-like toxicity (see above).

Regarding PBDE, deca-BDE (BDE-209) contributed on average about 13% to $\sum PBDE_7$. The contribution of the octa-brominated diphenyl ether (octa-BDE) BDE-197 and nona-BDEs-206, - 207 and -208 to the sum of 25 PBDE was in the

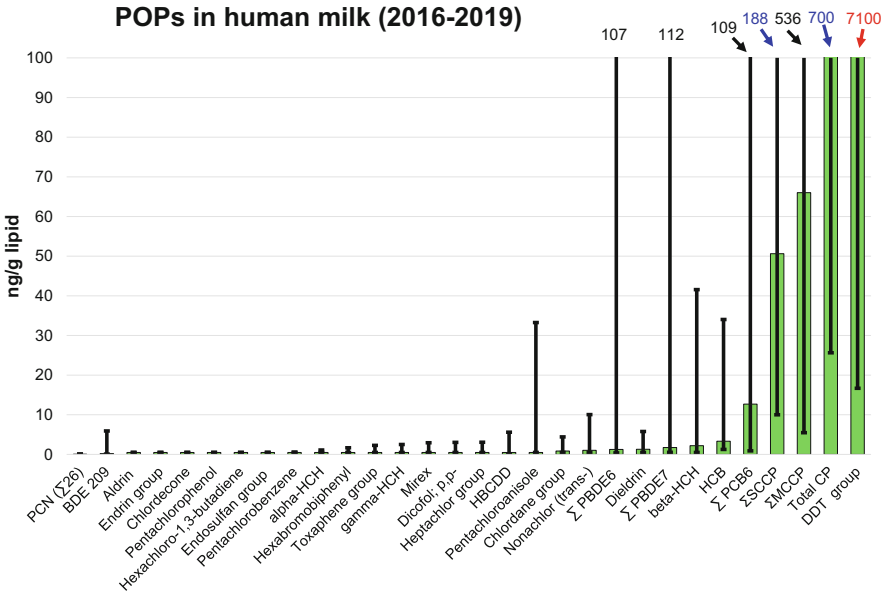

Fig. 13 Range of concentrations of lipophilic chlorinated and brominated POPs in human milk (ng/g lipid) from 43 countries in the period 2016–2019 (median with error bars indicating the minimum and maximum) (This figure is scaled to 100 ng/g lipid as maximum value allowing a visual comparison also at lower concentration ranges)

range of the six recommended analytes and BDE-209. Therefore, their addition to the list of recommended analytes should be considered (Schächtele et al. 2023b).

The range below 5 ng/g lipid can be seen as background concentrations of non-dioxin-like chlorinated and brominated POPs. Background concentrations are defined as that portion of the measured human milk levels that is found in the absence of specific sources and therefore is not attributable to a known exposure, e.g. to use of the chemical of interest or to emissions within the study area. In contrast to findings of high concentrations, e.g. after use of chemicals, and/or after a sufficient long withdrawal period (measures restricting their manufacture and use) for many POPs, the levels are described as "low background levels". However, the term "background level" does not imply per se any level of safety. With respect to potential adverse effects, risk assessments need to consider many factors, including the toxicity of the chemical of interest and the determined concentration range (van den Berg et al. 2016). Further, due to observed levels, targeted research should look at the balance among potential adverse effects against positive health aspects for the breastfed infants for three groups of POPs (dioxin-like compounds; non-dioxin-like chlorinated and brominated POPs; PFAS) regarding potentially needed updates of the WHO guidance.

The relative share of SCCP and MCCP concentrations in addition to remaining 28 chlorinated and brominated analytes as listed in 2019 is illustrated by Fig. 14

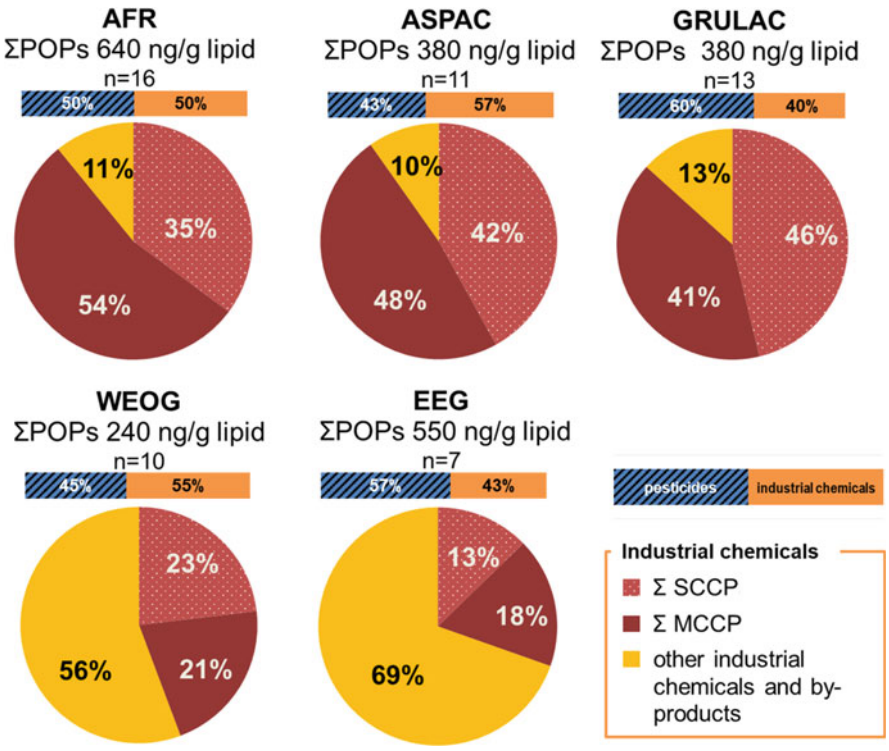

Fig. 14 Median sum of all POPs analysed in pooled human milk samples from 2015–2019, sorted by UN regions and broken down into Stockholm Convention POPs groups (bar charts) and further into components of the "industrial chemicals" POPs group (pie charts). AFR: Africa, ASPAC: Asia-Pacific Group, EEG: Eastern European Group, GRULAC: Group of Latin American and Caribbean Countries, WEOG: Western European and Others Group

(Krätschmer et al. 2023). The Stockholm Convention on POPs was sorted into two groups:

- Pesticide group: aldrin, chlordane, chlordecone, DDT, dicofol; dieldrin, endosulfan, endrin, heptachlor, alpha-hexachlorocyclohexane (HCH), beta-HCH; gamma-HCH; mirex, pentachlorobenzene, pentachlorophenol (including pentachloroanisole) and toxaphene
- (Other) Industrial chemicals and by-products: hexabromobiphenyl (HBB), hexabromocyclododecane (HBCDD), HCB, hexachlorobutadiene; PBDE (tetra- and pentabromodiphenyl ether; hexa- and heptabromodiphenyl ether; decabromobiphenyl ether), PCB, PCDD, PCDF, and PCN

SCCP and MCCP (proposed to be listed to the Convention) dominated over the POPs grouped as industrial chemicals and by-products in the African, the Asia-Pacific and the Latin American and Caribbean Regional Groups. For European

countries, the contrary was observed—proportion of "other industrial POPs and by-products" (mainly PCB) in the sum of concentrations of all POPs was much higher than that of SCCP and MCCP, respectively.

Individual samples (from individual donors) can provide additional information on the distribution of exposures and on factors possibly contributing to such exposure. Compared to pooled samples, they can span a broad range of concentrations. If significantly elevated levels are found in pooled samples, a follow-up survey/monitoring is usually recommended; if levels are quite low, no particular additional effort would generally seem to be necessary. Given the design of the milk studies, i.e. analysis of pooled samples, the dominance of SCCP and MCCP in comparison to levels of most other POPs in UN Regional Groups gives cause for concern. If the pooled samples from mothers without any known major POPs contamination sources already show this consistent pattern with a dominance of CP, individual samples (e.g., from local population close to POPs emission sources, as a result of exposure to consumer products or from the domestic environment) might be even markedly higher. The lactational intake of SCCP and MCCP of the breastfed infant in the microgram scale resulting from the mothers' dietary and environmental background exposure should therefore motivate targeted follow-up studies and further regulatory efforts (or, in the case of MCCP, any, e.g. restriction in products) (Krätschmer et al. 2023).

5.3 Perfluoroalkyl Substances (PFAS)

As mentioned above, PFAS concentrations are reported on product basis and therefore cannot be compared with concentrations of lipophilic POPs determined on lipid basis. 44 samples from 42 countries collected in the period 2016–2019 were analysed for PFAS. Within this group, the PFOS concentrations were in a wide range of <6.2–212 pg/g fresh weight (fw) (median: 13.2 pg/g fw) and the PFOA concentrations in a quite narrow range of 6.2–37.4 pg/g fw (median: 15.8 pg/g fw). PFHxS concentrations in 40 samples were below LOQ (<5.5 pg/g fw); four samples had concentrations between 7.3 pg/g fw and 111 pg/g fw (Fiedler and Sadia 2021).

6 Overall Conclusions

The concept of the WHO UNEP-coordinated exposure studies with standardized protocols for preparation of pooled samples considered to be representative for a country or subpopulation in a country and analysis in designated Reference Laboratories provides reliable and comparable data for POPs for human milk. Such studies have been performed over three decades (starting in 1987 with the focus on PCDD/PCDF and PCB and expanding to cover all 30 POPs listed by the Stockholm Convention in 2019 and in addition two other candidate POPs proposed for listing).

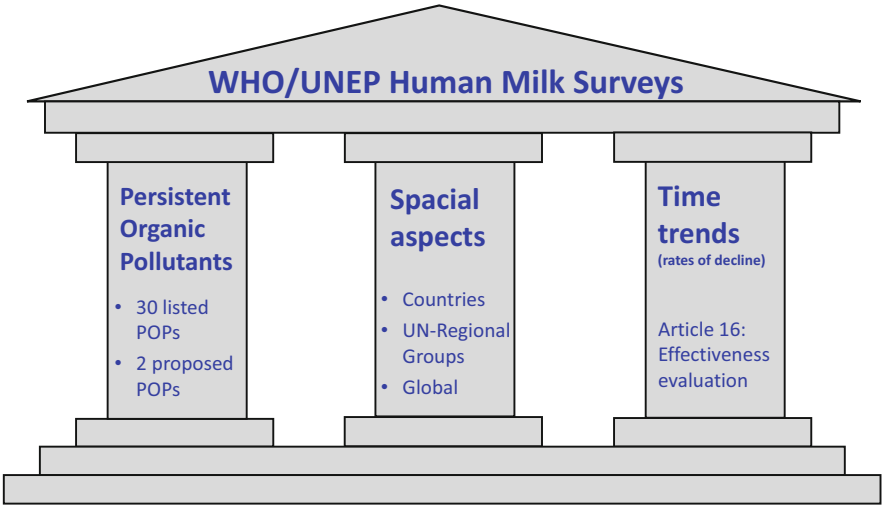

Fig. 15 Pillars of the WHO/UNEP-coordinated exposure studies

For discussion of country-specific results obtained for a certain analyte at a certain time, the three-dimensional picture can be derived by the assessments of the three pillars of this core matrix (Fig. 15): (1) the relative importance ("ranking") of the analytes of interest (30 listed and 2 candidate POPs proposed for listing as of 2019); (2) regional aspects (at the level of countries, UN Regional Groups or globally), and (3) time trends and their assessments for the effectiveness evaluation of the Stockholm Convention as requested by its Article 16.

The consideration of countries with repeated participation provides the best possible data set for the evaluation of temporal trends and for an assessment of the effectiveness of adopted measures. It is important to continue this existing monitoring arrangement in the same setting to secure sufficient data for reliable time trend assessments in the future. Due to the long half-life of POPs, only long-term trends can give an indication of the effectiveness of measures, and can identify possible problems to allow for targeted intervention.

Acknowledgements This publication was developed in the framework of the projects titled "Implementation of the POPs Monitoring Plan in the Asian Region" and "Continuing regional Support for the POPs Global Monitoring Plan under the Stockholm Convention in the Africa, Asia-Pacific and Latin-American and Caribbean Region", funded by the Global Environment Facility and in close collaboration with and support of CVUA Freiburg.

The worldwide implementation of the Global Monitoring Plan for POPs, including that of the WHO/UNEP global human milk studies, is made possible thanks to the generous contributions to the Stockholm Convention Voluntary Trust Fund by the Governments of Japan, Norway and Sweden and through the European Union's Global Public Goods and Challenges Programme (GPGC). Further, the substantial contributions made by the Global Environment Facility to support POPs monitoring activities in regions implemented by UNEP, in close collaboration with WHO, particularly for the global human milk studies, are greatly appreciated.

The authors express their gratitude to the National Coordinators of the WHO/UNEP-coordinated exposure surveys for their excellent work to collect the human milk samples and to prepare and send the pooled samples to the Reference Laboratories, which included great efforts to plan and implement the national studies with the assistance of the health, environment, laboratory, and administrative staff.

The authors thank Katarina Magulova and Ana Witt (Secretariat of the Basel, Rotterdam and Stockholm Conventions) and Haosong Jiao (UNEP Economy Division, Chemicals and Health Branch) for their support and contributions to these studies, and Heidelore Fiedler for the conception and implementation of the Global Monitoring Plan projects at her time at United Nations Environment Programme, Economy Division, Chemicals and Health Branch.

Disclaimer The authors alone are responsible for the views expressed in this publication, which do not necessarily represent the decisions, policy, or views of the World Health Organization and the United Nations Environment Programme.

Appendix

Regional Differentiation

Toxic equivalents of PCDD/PCDF and Dioxin-like PCB (WHO_{2005}-TEQ)

The range of concentrations of toxic equivalents (TEQ) of PCDD/PCDF and dioxin-like PCB (expressed as WHO_{2005}-TEQ; long term "WHO-PCDD/PCDF-PCB-TEQ [2005]", shortly "Total TEQ") in 232 pooled samples from 82 countries collected between 2000 and 2019 varies between 1.29 and 49 pg WHO_{2005}-TEQ/g, with a median of 7.24 pg/g. The highest median WHO_{2005}-TEQ concentrations were found in countries of the Eastern European Group and the Western European and Others Group with 12.0 pg/g and 10.3 pg/g, respectively. The widest variation was in Africa (range 1.29 to 49 pg/g). With median concentrations between 4 and 5 pg/g and maximum levels between 10 and 12 pg/g, the Pacific region in the Asia-Pacific Group and countries from the Latin American and Caribbean Group were at the lower end of the distribution (Fig. 16).

Time trends and changes in the fraction of regional groups over these periods have to be taken into consideration for this overall picture for a period of 20 years. Whereas in the 2000–2003 period, the majority of participants came from countries of the Eastern European Group and Western European and Others Group, in the 2016–2019 round, the majority came from the African Group, followed by the Group of Latin American and Caribbean Countries and then the Asia-Pacific Group. Figure 17 illustrates the regional differentiation (indicating the five UN Regional Groups by different colours) with temporal tendencies over the five rounds. In the 2000–2003 round, 21 of the 26 countries participating were from the Eastern European or Western European and Others Groups. In comparison to other regions, countries from these groups had quite high total TEQ concentrations (up to 27 pg WHO_{2005}-TEQ/g lipid in the pooled samples). In this period, Egypt had total TEQ concentrations comparable to European countries decreasing considerably until 2019. In the 2016–2019 round, four countries from the African Group and the

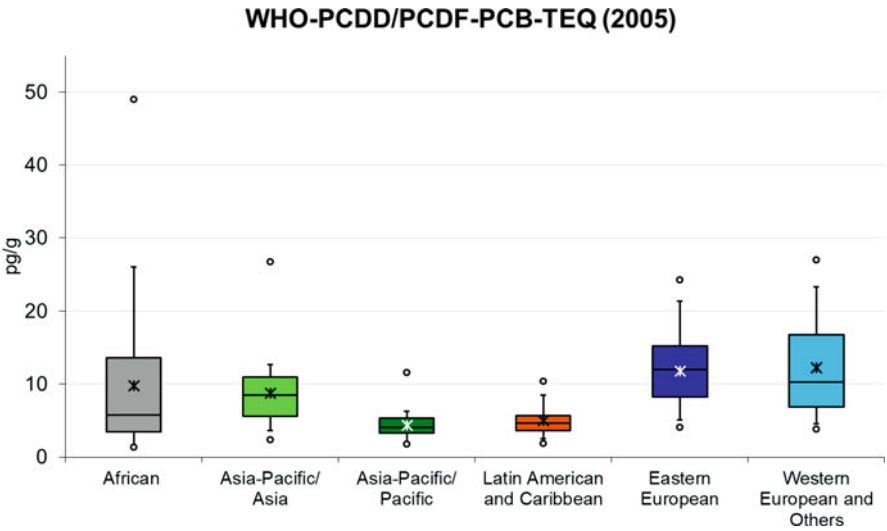

Fig. 16 Range of concentrations of total TEQ (WHO-PCDD/PCDF-PCB-TEQ [2005]) among UN regions (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands; pg/g lipid, $N = 232$ pooled samples from 82 countries) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

Marshall Islands are found at the upper end of the frequency distribution curve with concentrations between 6.7 and 11.6 pg/g. On the other side of the frequency distribution curve was Ethiopia with the lowest levels of total TEQ of all countries in the 2000–2019 studies (1.54 pg WHO_{2005}-TEQ/g in 2012 and 1.29 pg/g in 2019). For a detailed discussion, see Part III of this compendium (Malisch et al., 2023b).

Beta-HCH

As result of the metabolization of hexachlorocyclohexanes (HCH) in humans, the concentrations of alpha-HCH and gamma-HCH were in most human milk samples below the limit of quantification (<0.5 μg/kg lipid), whereas mainly beta-HCH accumulates in humans. Great differences of beta-HCH concentrations between UN Regional Groups and between countries in the same region were found, covering a range of three orders of magnitude with a minimum of <0.5 μg beta-HCH/kg lipid found in few countries and a maximum of 1020 μg beta-HCH/kg lipid found in 2002 in the Asia-Pacific Group. On average (as median and mean), the highest beta-HCH concentrations were found in the Eastern European Group (median 25.1 μg/kg lipid, mean 95.2 μg/kg lipid) with a range up to 476 μg/kg lipid (Fig. 18).

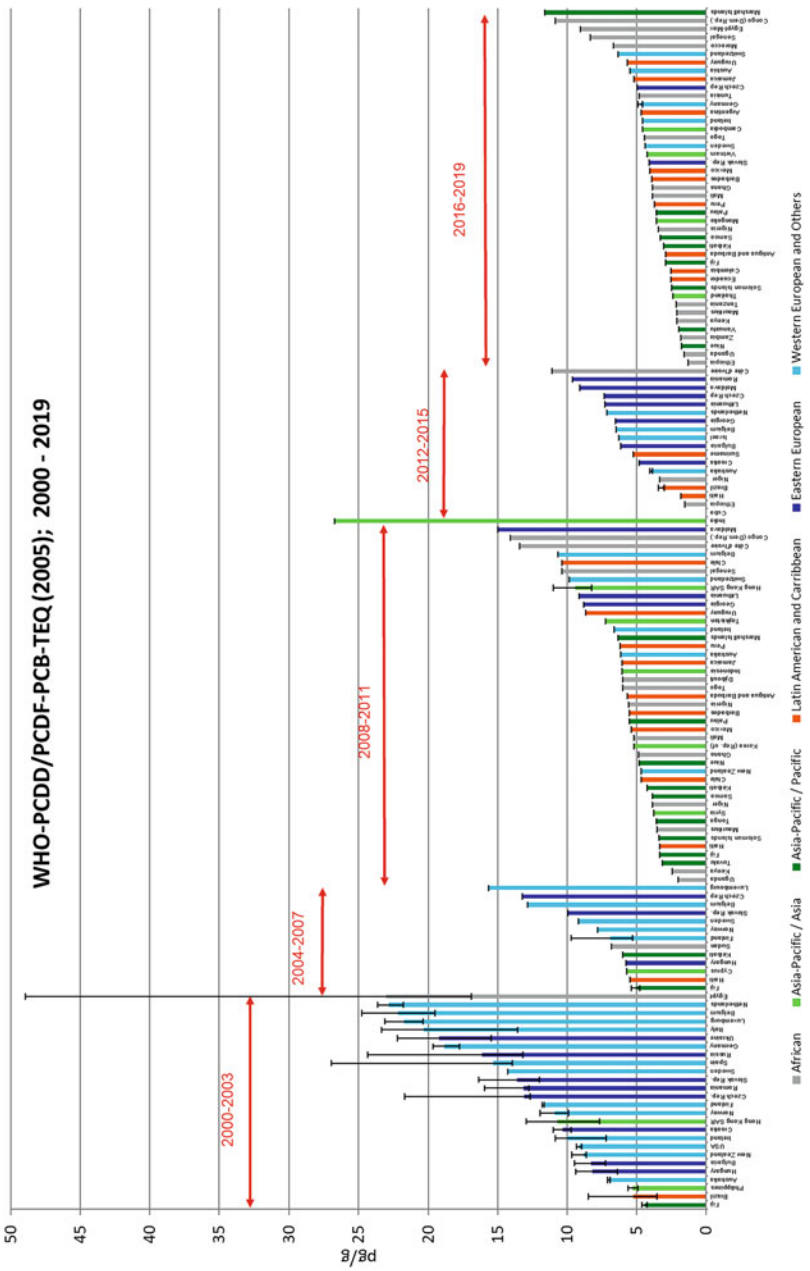

Fig. 17 Results of the 2000–2019 surveys for total TEQ in 232 pooled human milk samples from 82 countries of the five UN Regional Groups (with split of the Asia-Pacific Group into the subgroups Asia and Pacific Islands) indicated by different colours and classified into five periods of sample submission (pg WHO-PCDD/PCDF-PCB-TEQ [2005])/g lipid). Whiskers indicate the range of concentrations found in samples around the median concentration, if two or more samples were submitted by a country in a certain period

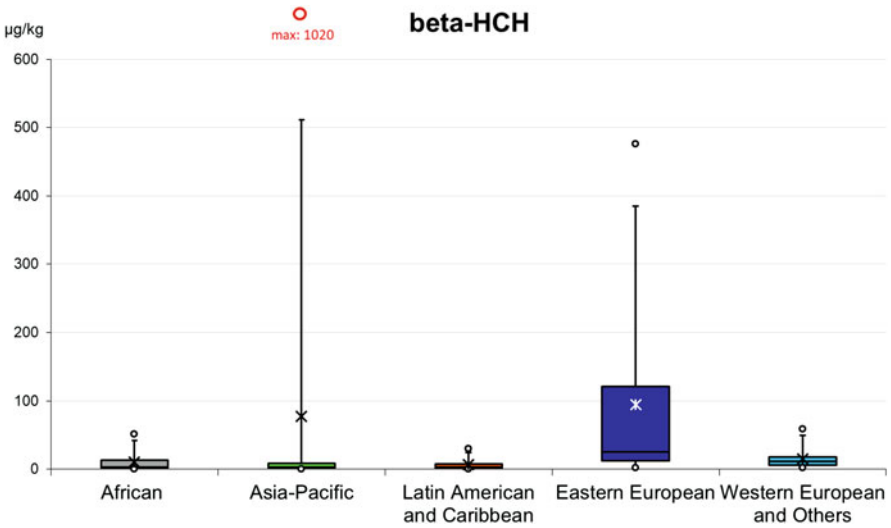

Fig. 18 Range of concentrations of beta-HCH in human milk among UN regions (µg/kg lipid; $N =$ 134 country results [with median, if results for two or more pooled samples are available in a certain period] from 82 countries, comprising the five periods from 2000 to 2019) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

The findings of high beta-HCH concentrations in different periods were influenced by the variation of countries participating in a certain UN region in a certain period. The highest concentration was found in 2002 in in Hong Kong SAR of China (1020 µg beta-HCH/kg lipid as the median of 10 pooled samples). Hong Kong participated twice, with a considerable decrease to the 2009 level of 290 µg beta-HCH/kg lipid (median of 4 samples from different population groups). A decrease was also found in countries of the Eastern European Group with repeated participation, e.g. the maximum level of 476 µg beta-HCH/kg lipid found in 2009 in Moldova decreased considerably to 150 µg beta-HCH/kg lipid in 2015 (Fig. 19). For a detailed discussion, see Part III of this compendium (Malisch et al., 2023c).

HCB

The maximum levels found for hexachlorobenzene (HCB) and therefore the ranges were much lower than for DDT and beta-HCH, with a minimum of about 1–2 µg/kg lipid found in some countries and a maximum of 154 µg/kg lipid found in 2009 in an Eastern European country. The median of 134 country results was 5.1 µg/kg lipid. All countries from Africa were at all times in this low background range below 5 µg/kg lipid, and many countries from the Pacific Islands and Latin America and the Caribbean were as well (Fig. 20).

In the Eastern European Group, a wide range of concentrations was observed between the background contamination as found in Croatia, 2014 (7.6 µg/kg lipid) and Bulgaria, 2014 (7.7 µg/kg lipid), in comparison to 154 µg/kg found in Moldova,

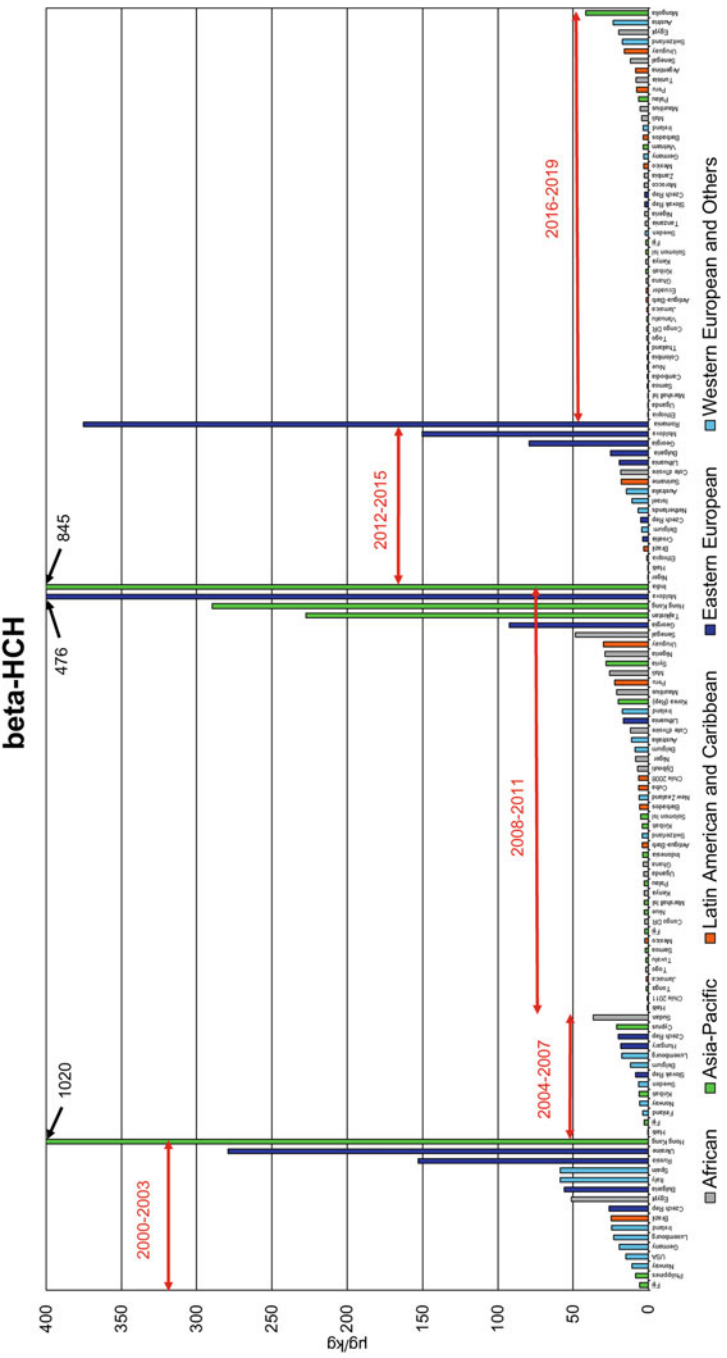

Fig. 19 Concentrations of beta-HCH in human milk (µg/kg lipid) from 82 countries of the five UN Regional Groups for five periods from 2000 to 2019 (N = 134 country results [with median, if results for two or more pooled samples are available in a certain period])

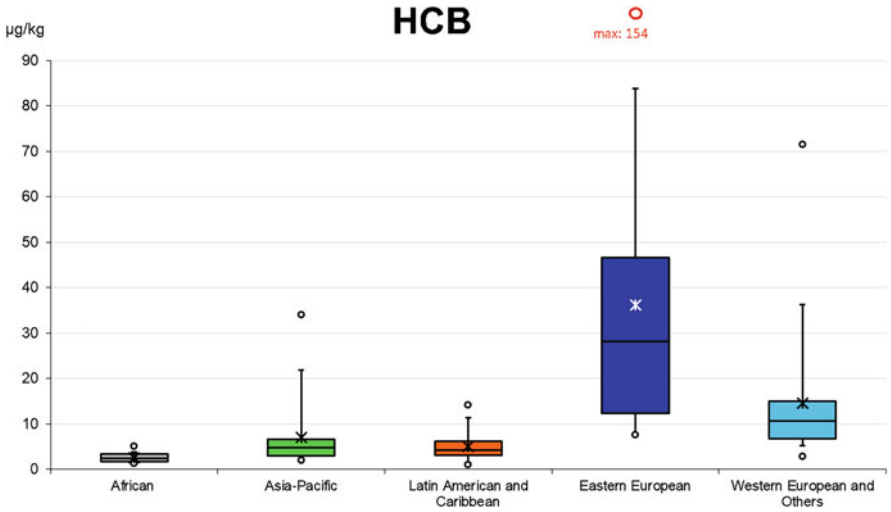

Fig. 20 Range of concentrations of HCB in human milk among UN regions (µg/kg lipid; $N = 134$ country results [with median, if results for two or more pooled samples are available in a certain period] from 82 countries, comprising the five periods from 2000 to 2019) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

2009. In Moldova, these concentrations decreased considerably to 46.7 µg HCB/kg lipid in 2015. The Czech Republic participated four times between 2000 and 2019; here a continuous downtrend is found from 76 µg/kg lipid in 2001 to 12.5 µg/kg lipid in 2019. Also in Western European countries, a gradual downward trend was observed from participation of countries in the 2000–2003 period until the 2016–2019 period (Fig. 21). For a detailed discussion, see Part III of this compendium (Malisch et al., 2023c).

Dieldrin

In the analyte group "Organochlorine pesticides and industrial contaminants", maximum concentrations above 20 µg/kg lipid were found for DDT, beta-HCH, HCB, and dieldrin. However, the dieldrin concentrations were considerably lower than those for the other three POPs. A maximum of 37.8 µg/kg lipid was found in a sample of the Asia-Pacific Region (sample from Tajikistan, 2009). The median concentration was between 1 and 2 µg/kg lipid in all UN Regional Groups except the Western European and Others Group (median 3.57 µg/kg lipid) (Fig. 22). Based on 134 country results, nearly 90% of the samples had dieldrin levels in a low background range below 5 µg/kg lipid (Fig. 23). For a detailed discussion, see Part III of this compendium (Malisch et al., 2023c).

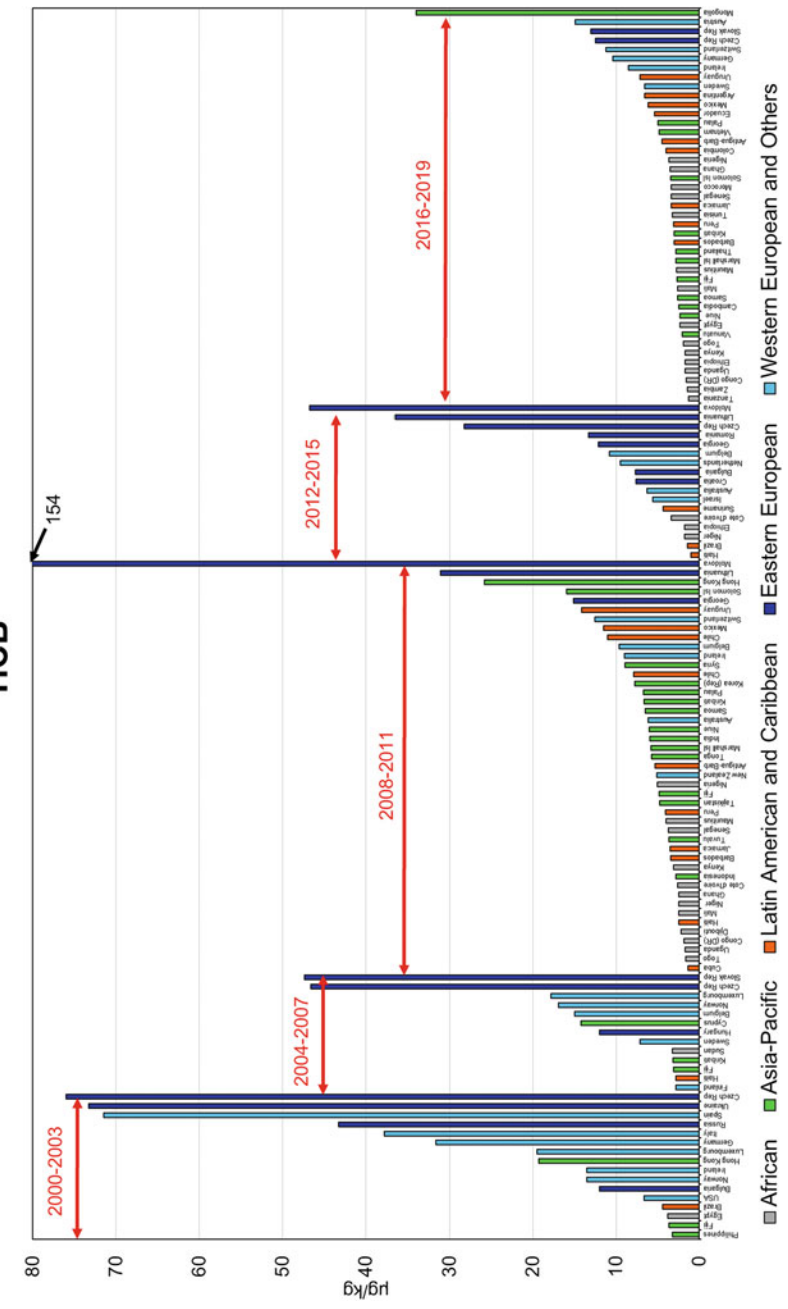

Fig. 21 Concentrations of HCB in human milk (μg/kg lipid) from 82 countries of the five UN Regional Groups for five periods from 2000 to 2019 (N = 134 country results [with median, if results for two or more pooled samples are available in a certain period])

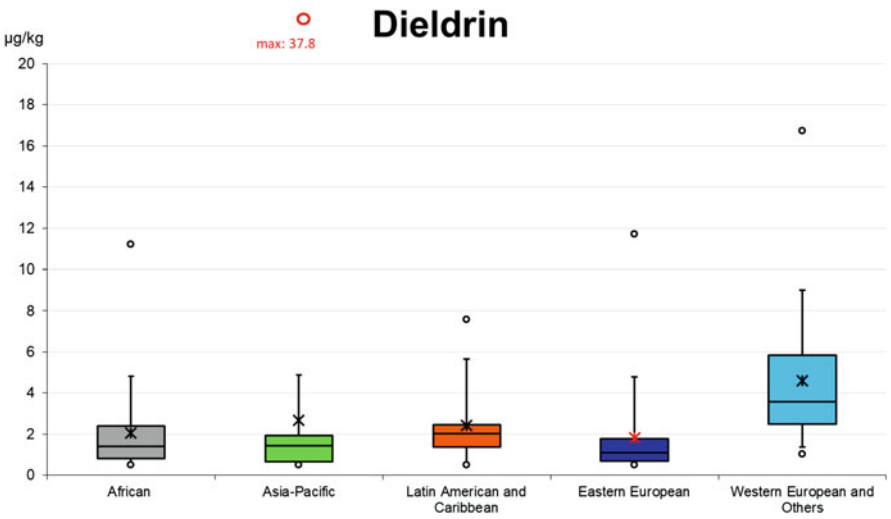

Fig. 22 Range of concentrations of dieldrin in human milk among UN regions (µg/kg lipid; $N = 134$ country results [with median, if results for two or more pooled samples are available in a certain period] from 82 countries, comprising the five periods from 2000 to 2019) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

PBDE

Large differences in levels of 124 country results (using the median, if results for two or more pooled samples are available in a certain period) from 80 countries collected between 2000 and 2019 were found for the sum of six polybrominated diphenyl ethers (PBDE) (BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183) (Figs. 24 and 25). 80% of these samples were in a range below 5 ng $\sum$ $PBDE_6$/g lipid, including all samples from Africa and the Eastern European Group. The highest concentration of 223 ng $\sum$ $PBDE_6$/g lipid was found in the 2000–2003 period in the Western European and Others Group (comprising Australia, Israel, New Zealand, and USA as "Others") in the USA, followed by 107 ng $\sum$ $PBDE_6$/g lipid in the Asia-Pacific Group in the 2016–2019 period (Marshall Islands). A closer look into highest country result found in the USA in 2003 reveals that the 223 ng $\sum$ $PBDE_6$/g lipid was calculated as median of two pooled samples from North Carolina [92.6 ng/g lipid] and California [352 ng/g lipid]. This was about three orders of magnitude above the lowest concentration found in 2009 in Syria (0.28 ng $\sum$ $PBDE_6$/g lipid). For a detailed discussion, see Part III of this compendium (Schächtele et al., 2023b).

PFOS

The range of concentrations of perfluorooctane sulfonic acid (PFOS) in 86 pooled samples from 59 countries collected between 2008 and 2019 varies between

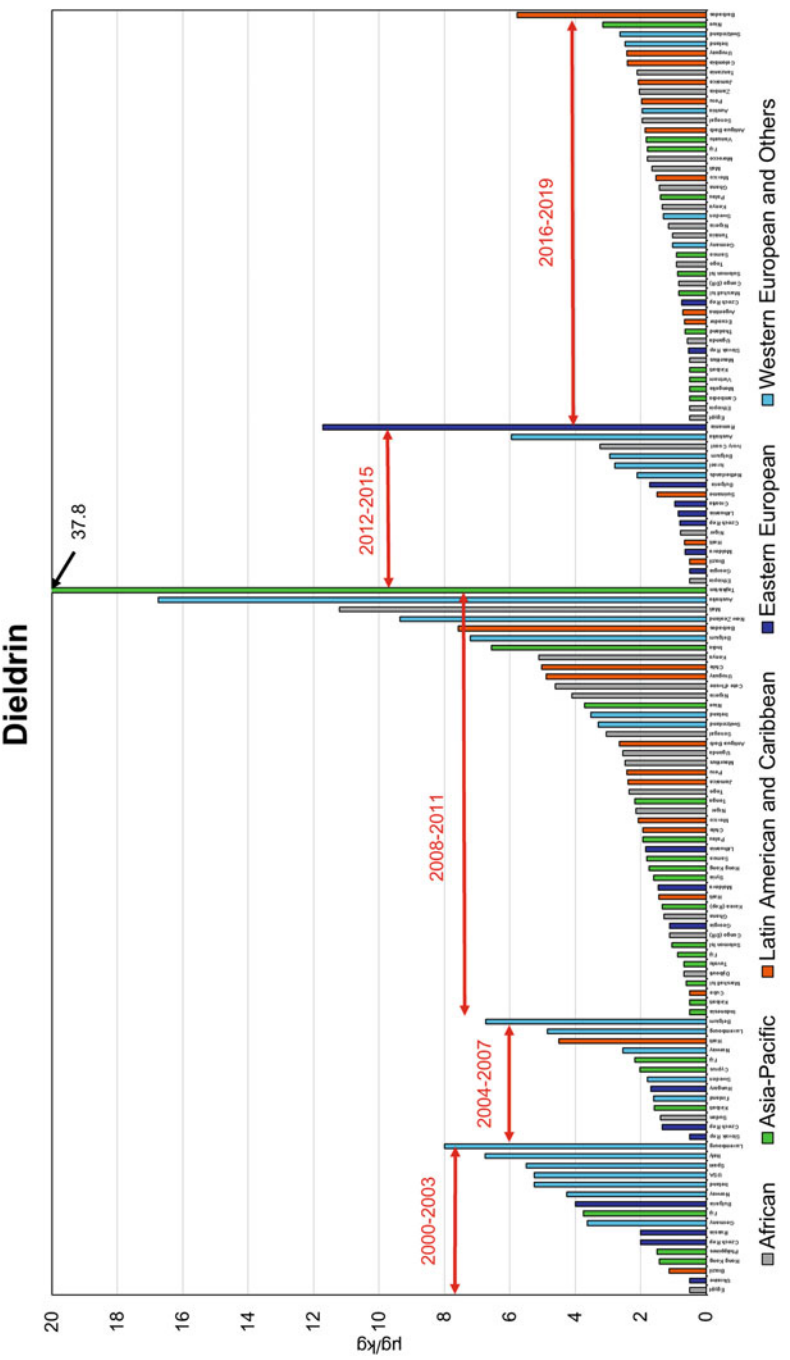

Fig. 23 Concentrations of dieldrin in human milk (µg/kg lipid) from 82 countries of the five UN Regional Groups for five periods from 2000 to 2019 (N = 134 country results [with median, if results for two or more pooled samples are available in a certain period])

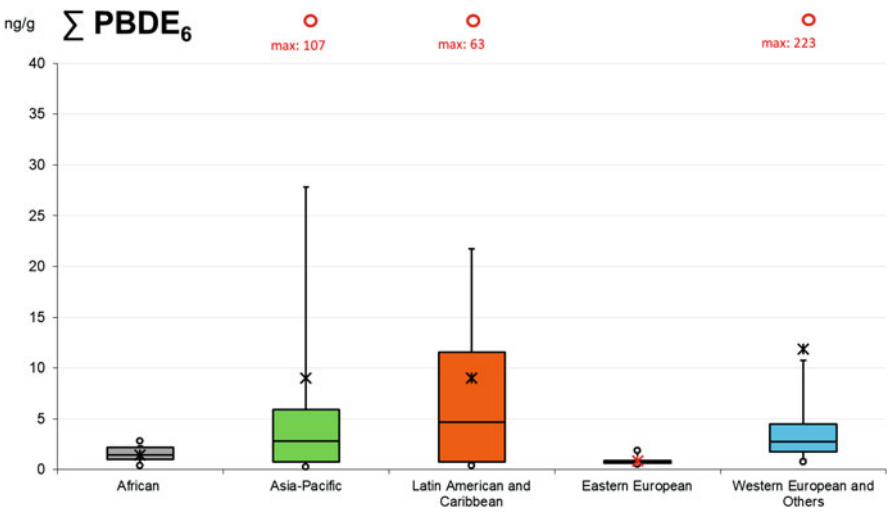

Fig. 24 Range of concentrations of ∑ $PBDE_6$ in human milk among UN regions (ng/g lipid; $N =$ 124 country results [with median, if results for two or more pooled samples are available in a certain period] from 80 countries, comprising the five periods from 2000 to 2019) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

<6.4 ng/l and 218 ng/L total PFOS (∑PFOS), calculated as sum of linear PFOS (L-PFOS) and branched isomers (br-PFOS), with median concentrations between 14 ng/L in the African region and 34 ng/L in the Eastern European region (Fig. 26). Most samples originated from the African region ($N = 27$), the fewest from the Western European and Others Group ($N = 8$; with no sample from the 2008–2011 period). The maximum concentration of 218 ng ∑PFOS/L was found in a sample from Kiribati collected in the 2016–2019 period. The range of concentrations found in 27 samples of the 2008–2011 period, in 15 samples of the 2012–2015 period, and 44 samples of the 2016–2019 period is illustrated in Fig. 27 with identification of the UN Regional Groups in different colours. For a detailed discussion including the presentation of results for other perfluorinated alkane substances (PFAS), in particular perfluorooctanoic acid (PFOA; listed in 2019) and perfluorohexane sulfonic acid (PFHxS; proposed for listing as of 2019), see Fiedler and Sadia (2021) and Fiedler et al. (2022).

Chlorinated Paraffins

Chlorinated paraffins (CP) are very complex mixtures of several million theoretically possible compounds. Contrary to medium-chain CP (MCCP, C_{14}-C_{17}), short-chain chlorinated paraffins (SCCP, C_{10}-C_{13}) have been listed in 2017 in Annex A (Elimination) of the Stockholm Convention on Persistent Organic Pollutants. The concentrations of CP were determined in 84 pooled human milk samples collected between 2009 and 2019 in 57 countries (Krätschmer et al., 2023). Until 2015, only

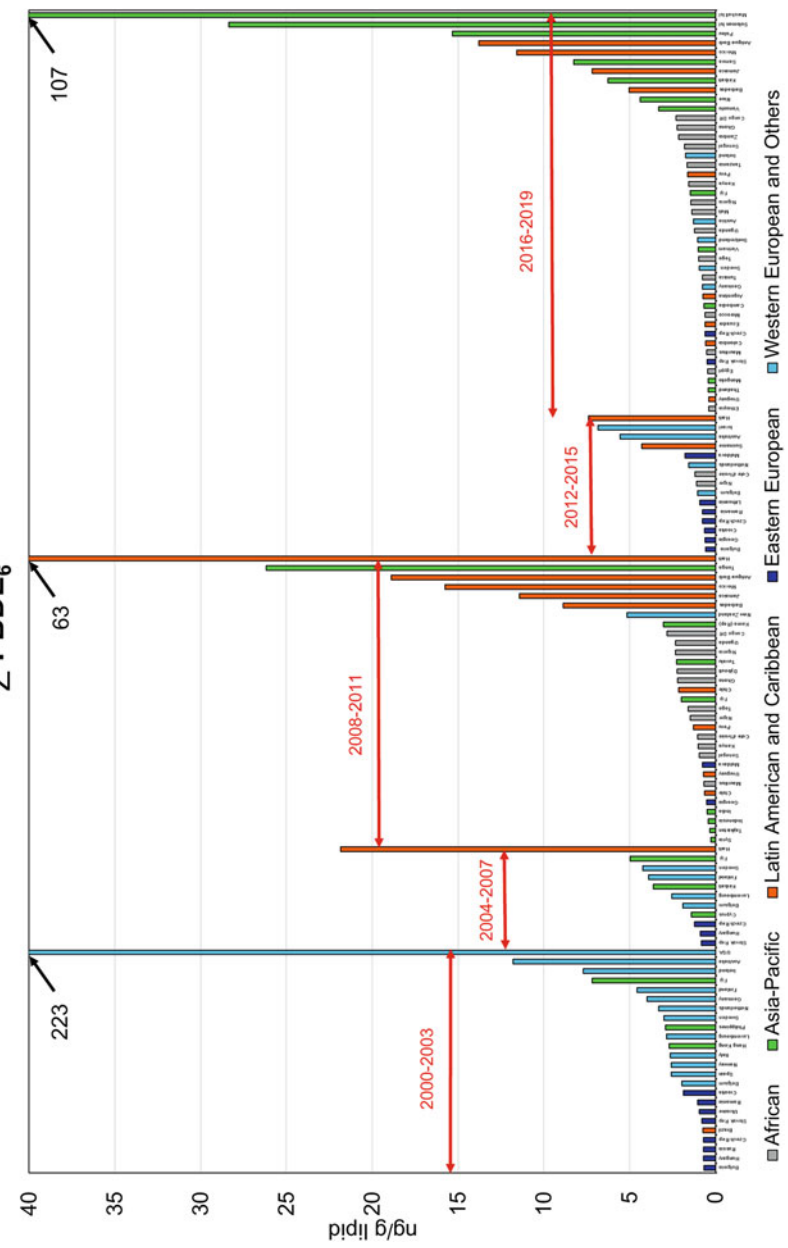

Fig. 25 Concentrations of $\sum$ $PBDE_6$ in human milk (ng/g lipid) from 80 countries of the five UN Regional Groups for the five periods from 2000 to 2019 (N = 124 country results [with median, if results for two or more pooled samples are available in a certain period])

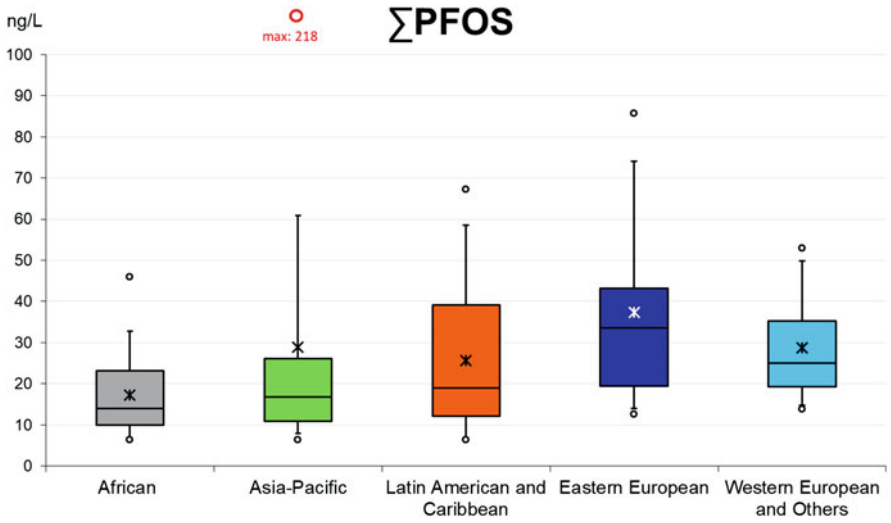

Fig. 26 Range of concentrations of ∑PFOS in human milk among UN regions (ng/L; $N = 86$ pooled samples from 59 countries, comprising the period from 2008 to 2019) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

total CP content was determined. Thus, for 27 samples of the 2009 to 2011 period and some samples of the 2012 to 2013 period, only the sum of CP is available. Later, a new quantification method was developed able to differentiate between SCCP and MCCP and to give information on homologue group patterns. Some samples of the period 2012 to 2013 and all samples arriving after 2014 were analysed with this new quantification method, altogether 57 samples. Therefore, the sum of all detected CP is used as parameter to present all analysed samples here. CP were present in all 84 samples, ranging 8.7–700 ng/g lipid. On average, African and Asia/Pacific pooled samples had the highest combined SCCP and MCCP content of the five UN regional groups: With a median of 160 ng/g lipid each, they surpassed median levels of the other three groups by a factor of 2–3 (Fig. 28).

As up to 2022 only SCCP were listed, the relation between SCCP and MCCP is important for interpretation of the sum parameter. In most pooled country samples of all UN regional groups, MCCP concentrations at least equalled SCCP concentrations, contributing 24–85% to the total CP levels reported here (Fig. 29). In 36 of the 53 countries where distinct data is available, MCCP even surpassed SCCP. Using the SCCP/MCCP ratio as an indication of this relation, it is interesting to see that African and Eastern European country samples had a tendency towards a higher dominance of MCCP, whereas in the other UN regional groups, median SCCP levels were closer to equal distribution compared to MCCP (African group 58% MCCP, Asian-Pacific Group 55%, Group of Latin America and Caribbean Countries 53%, Eastern European Group 67%, Western European and Others Group 54%).

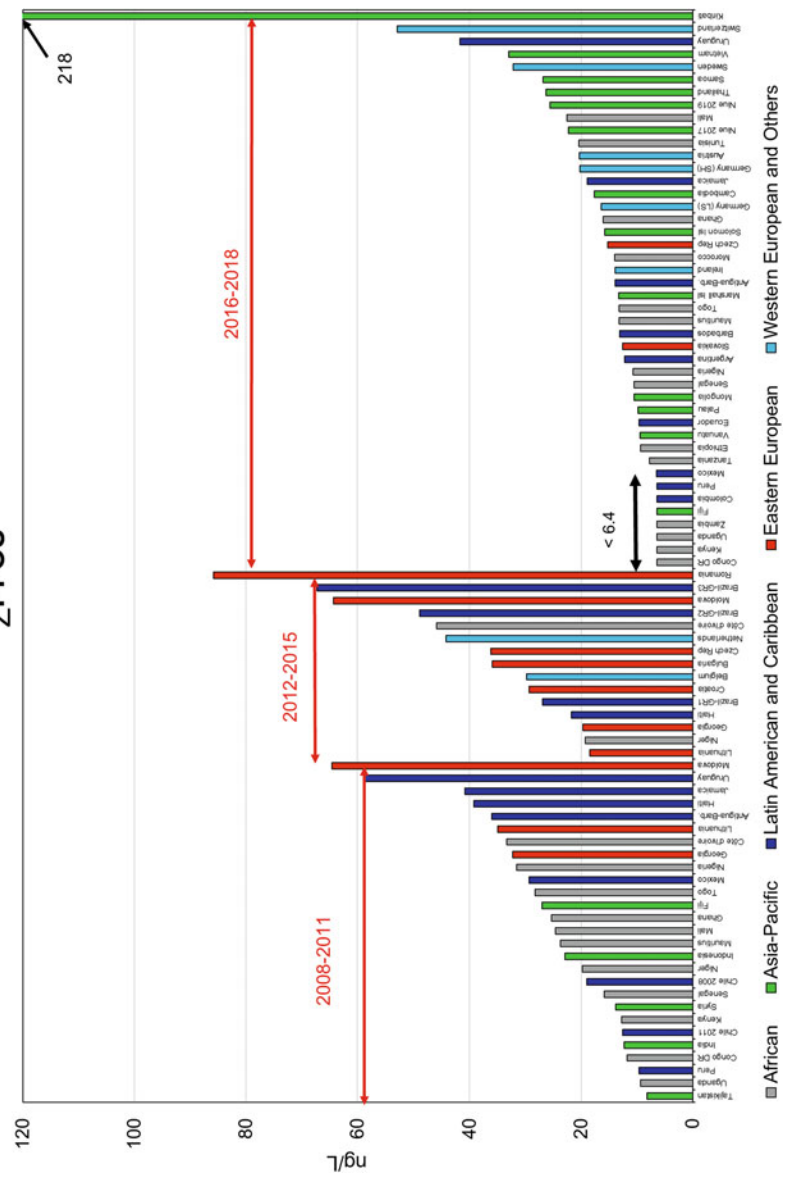

Fig. 27 Concentrations of ∑PFOS in human milk (ng/L) from 59 countries of the five UN Regional Groups for three periods between 2008 and 2019 (N = 86 pooled samples)

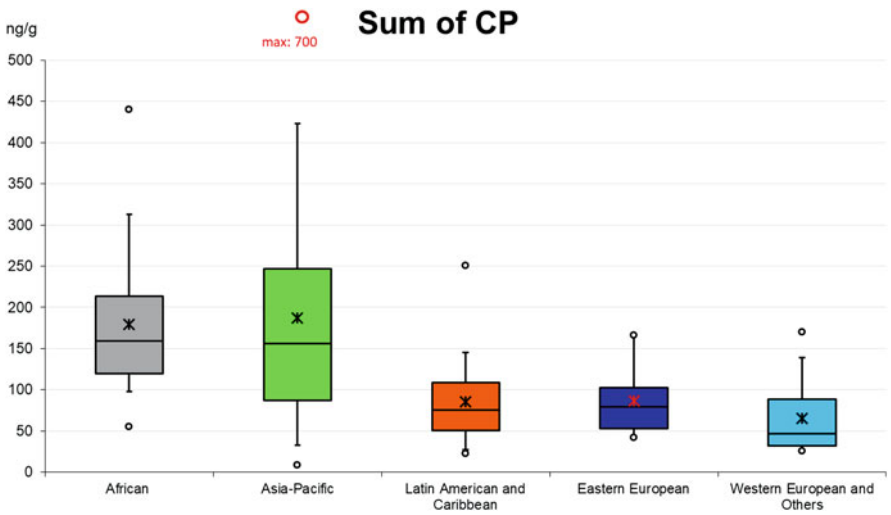

Fig. 28 Range of concentrations of sum of CP in human milk among UN regions (ng/g lipid; $N = 84$ samples from 57 countries, comprising the 2009–2019 period) [Box plot; minimum and maximum: as circles; 5th and 95th percentile: as whiskers; lower (25–50%) and upper (50–75%) quartiles, separated by the line for the median: as box; mean: as asterisk]

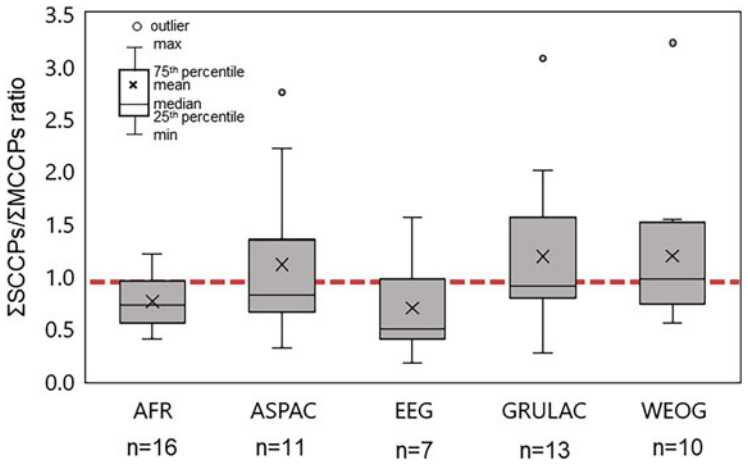

Fig. 29 Range of SCCP/MCCP ratios, sorted by UN Regional Groups. The dotted horizontal line indicates ratio = 1, i.e. equal presence of SCCP and MCCP. *AFR* African Group, *ASPAC* Asian-Pacific Group, *EEG* Eastern European Group, *GRULAC* Group of Latin America and Caribbean Countries, *WEOG* Western European and Others Group

Figure 30 illustrates the differences in levels of 84 pooled samples from 57 countries collected between 2009 and 2019 for the sum of CP. Most notably,

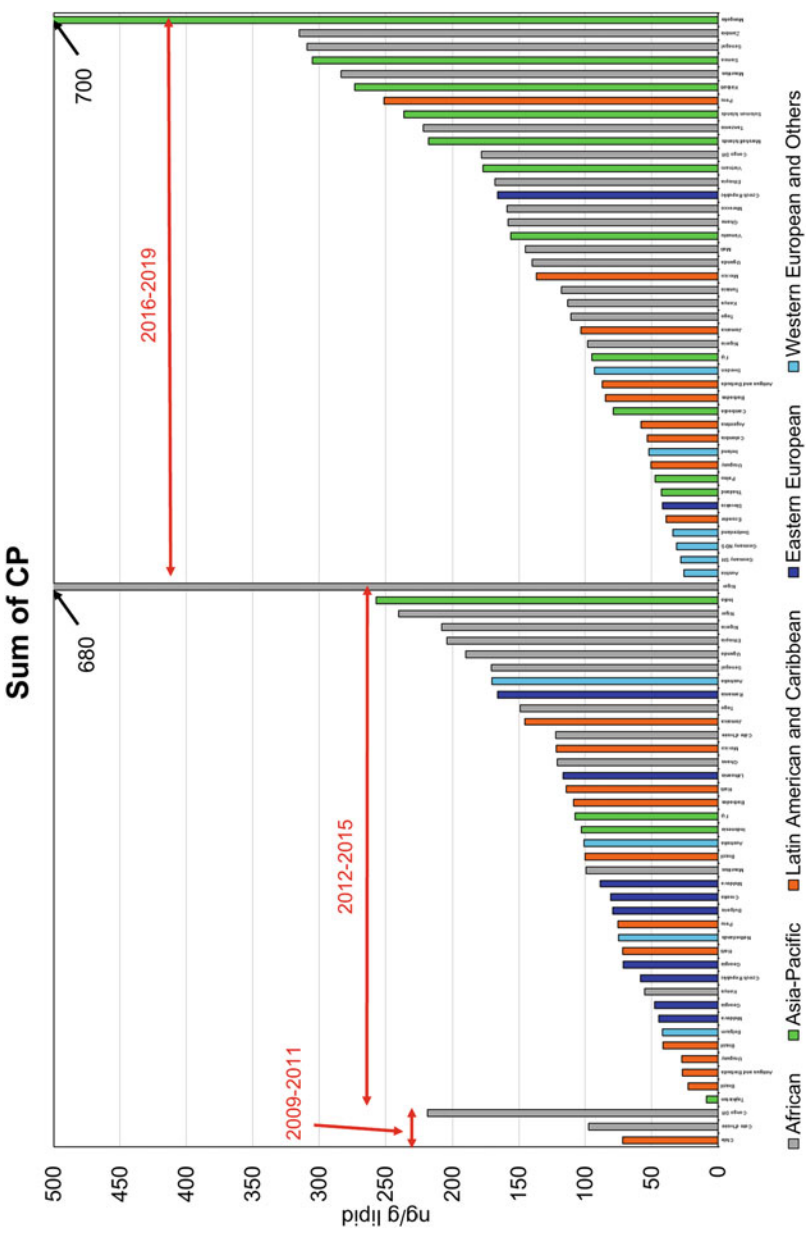

Fig. 30 Concentrations of sum of CP in human milk (ng/g lipid) from 58 countries of the five UN Regional Groups for three periods between 2009 and 2019 ($N = 84$ samples)

the samples from Mongolia (2018, 700 ng CP/g lipid) and Niger (2015, 680 ng CP/g lipid) were higher than other samples from their regional groups. For a detailed discussion, see Part III of this compendium (Krätschmer et al., 2023).

Assessments of Time Trends

Beta-HCH

Conclusions on time trends for beta-HCH in the different UN regions cannot easily be drawn. As shown already for DDT in subsection 4.1, also the time trends of the *median* beta-HCH concentrations in the UN regions are not consistent. For the median of the samples in all UN regions, a decrease of 91% was found from the 2000–2003 period (25.3 μg/kg lipid) to the 2016–2019 period (2.4 μg/kg lipid). A considerable decrease is also seen in the individual UN regions if the median of the beta-HCH concentrations of the 2000–2003 period is compared to the 2016–2019 period. However, in the three rounds in-between, considerable variations were observed, notably in Asian and in the Eastern European countries starting with an initial downward trend from 2000–2003 to 2004–2007. This, however, is followed by an increase in the period 2008–2011 before the concentrations decrease again to 2016–2019. Other than for DDT, the temporal trends using the *mean* concentrations look quite comparable to the trends derived from the median with no significant maxima in other periods (Malisch et al., 2023c).

The evaluation only of countries with repeated participation shows decreasing tendencies in nearly all countries. Figure 31 illustrates the temporal tendencies in 44 countries of the five UN regional groups that participated two or more times. In nearly all countries, decreasing tendencies are observed, with a median of 65% decrease over the years between first and last participation. As for DDT, a differentiation of levels above or in the range of background contamination seems to be advised. If high levels are found, sources might be detected which could be eliminated, whereas at low background levels, other factors cannot be influenced. Therefore, in some countries a variation at low background levels (<5 μg/kg) over time is observed when concentrations tend to level out.

An overall decrease within a 10-year period between 50% and 98% was achieved for beta-HCH levels in all the UN regions and at a global level. The overall decrease per 10 years calculated by the Theil–Sen method for exponential trends and the additionally applied median method to derive time trends were quite comparable in nearly all UN regions and at the global level (Table 8). On a global level and in all UN regions except Latin America and the Caribbean, all trends were significant (p-value <0.001 in three regions and globally, 0.037 in Eastern Europe). Globally, a decrease per 10 years by 84% was found for beta-HCH (calculated by the Theil–Sen method). For more details, see Malisch et al. (2023e).

HCB

In general, concentrations of HCB show a downward trend globally: The *median* of all samples decreases from the 2000–2003 period (16 countries, 16.4 μg/kg lipid) to

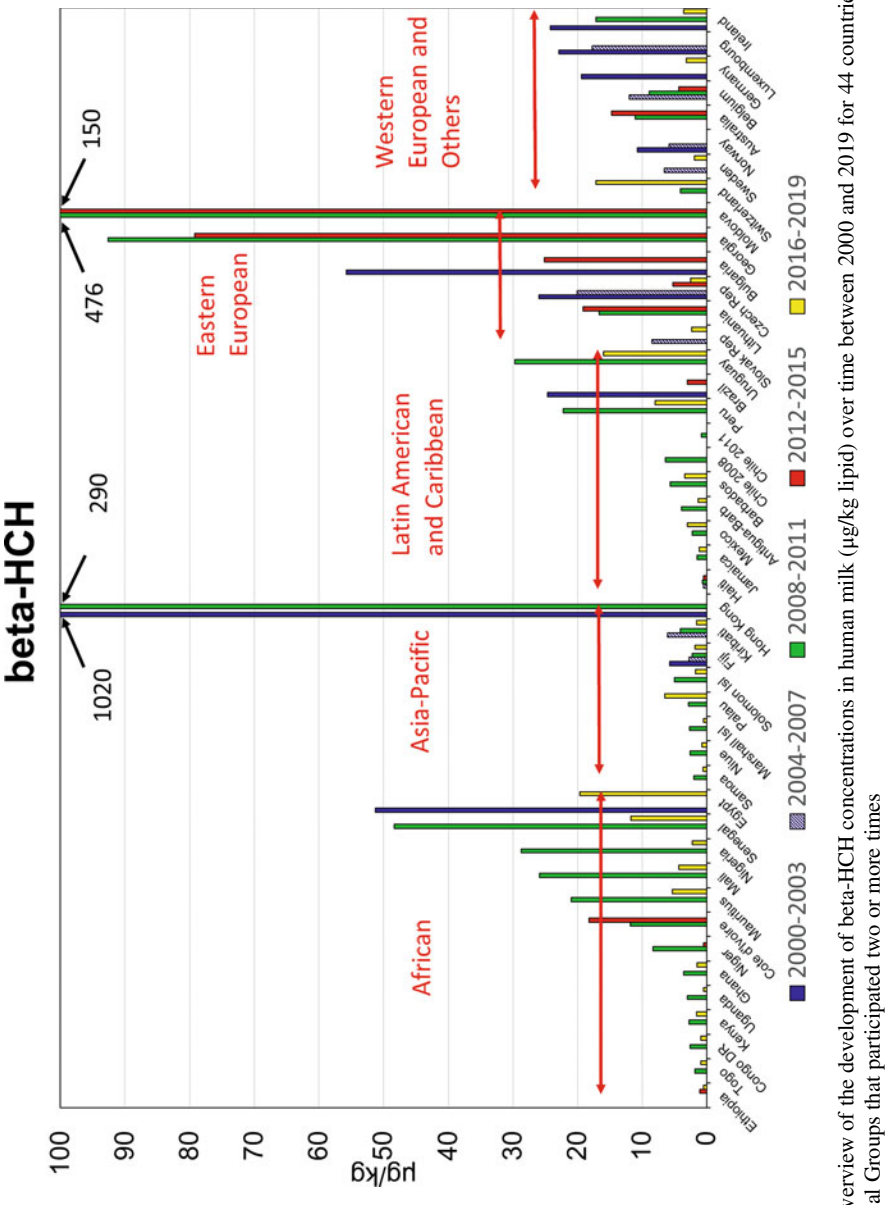

Fig. 31 Overview of the development of beta-HCH concentrations in human milk (μg/kg lipid) over time between 2000 and 2019 for 44 countries of the five UN Regional Groups that participated two or more times

Table 8 Overall decrease (%) of beta-HCH concentrations in human milk (expressed as μg beta-HCH/kg lipid) in the five UN Regional Groups and worldwide (computed using all samples submitted by countries with repeated participation)

UN Regional Group	N of countries	Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	
African	13	82.5	78.4	<0.001
Asian-Pacific	8	97.5	76.1	<0.001
Latin American and Caribbean	9	51.9	48.0	0.105
Eastern European	6	63.4	55.5	0.037
Western European and Others	8	62.9	65.8	<0.001
Global	44	83.5	63.4	<0.001

the 2016–2019 period (43 countries, 3.3 μg/kg lipid) by about 80%. Of particular interest are time trends in regions with higher initial concentrations. In the Eastern European Group, the median HCB concentration falls continuously from the 2000–2003 period (58.3 μg/kg lipid) to the 2012–2015 period (13.3 μg/kg lipid) and then levels out around 13 μg/kg lipid in the 2016–2019 period. In countries of the Western European and Others Group, a downward trend is also observed from the 2000–2003 period (19.5 μg/kg lipid) to the 2008–2011 period (9.0 μg/kg lipid) and then leveling out at about 10 μg/kg lipid. In other groups, the background contamination levels out at concentrations up to 5 μg HCB/kg lipid (limit of quantification: 0.5 μg/kg lipid). The picture using the *mean* concentrations instead of median looks as if there was a maximum in the Eastern European Group in the 2008–2011 period. However, this is caused by a high HCB concentration found in the pooled sample of one country submitted at that time (sample from Moldova, 2009, 154 μg/kg lipid) (Malisch et al., 2023c).

The evaluation only of countries with repeated participation shows decreasing tendencies in nearly all countries. Figure 32 illustrates the temporal tendencies in 44 countries of the five UN regional groups that participated two or more times. In most countries, decreasing tendencies are observed, with a median of 27% decrease over the years between first and last participation. However, as explained above, also for HCB a variation at low background levels (<5 μg/kg) over time is observed when concentrations tend to level out.

In the African Group with all pooled samples collected over time being in the background range of or below 5 μg HCB/kg lipid, the overall decrease within 10 years was 11% calculated by the Theil–Sen method. Overall decrease rates between 30% and 67% were calculated by the Theil–Sen method for the other UN Regional Groups, and 57% on a global level (Table 9). For more details, see Malisch et al. (2023e).

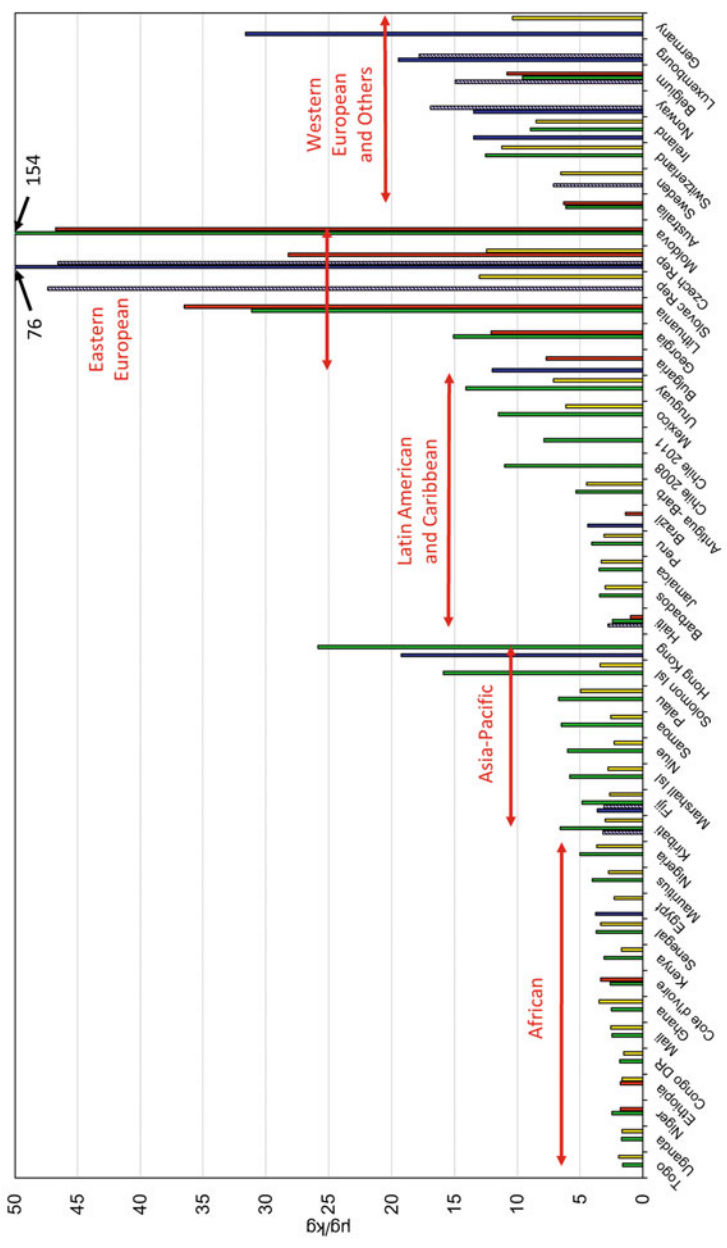

Fig. 32 Overview of the development of HCB concentrations in human milk (μg/kg lipid) over time between 2000 and 2019 for 44 countries of the five UN Regional Groups that participated two or more times

Table 9 Overall decrease (%) of the HCB concentration in human milk (expressed as µg/kg lipid) in the five UN Regional Groups and worldwide (computed using all samples submitted by countries with repeated participation)

UN Regional Groups	*N* of countries	Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	
African	13	11.3	10.2	0.002
Asian-Pacific	8	67.2	49.1	<0.001
Latin American and Caribbean	9	30.7	49.6	0.037
Eastern European	6	46.6	51.2	<0.001
Western European and Others	8	44.8	17.3	<0.001
Global	44	56.7	44.8	<0.001

PBDE

As shown above (Sect. 7.1.5), a considerable variation of the PBDE concentrations in human milk from 80 countries in the five periods between 2000 and 2019 was observed, depending on the inclusion of individual countries in a certain round. Of all samples, 80% were in a range below 5 ng $\sum$ $PBDE_6$/g lipid; the highest concentration of 223 ng $\sum$ $PBDE_6$/g lipid was found in the 2000–2003 period in the Western European and Others Group, followed by 107 ng $\sum$ $PBDE_6$/g lipid in the Asia-Pacific Group in the 2016–2019 period. A **general estimation of time trends** is quite difficult. In particular in the Asia-Pacific Group, the Group of Latin American and Caribbean Countries and in the Western European and Others Group, median or maximal concentrations don't appear to have a continuous downward time trend. The median of all country results shows a decrease of about 50% from the 2.62 ng $\sum$ $PBDE_6$/g lipid in 2000–2003 period to 1.38 ng $\sum$ $PBDE_6$/g lipid in the 2016–2019 period. These are seen as current background levels (Schächtele et al., 2023b).

Figure 33 illustrates the temporal tendencies in 36 countries of the five UN regional groups that participated two or more times. In most countries, a fluctuation at background levels (<5 ng/g lipid) over time is observed when concentrations tend to level out. However, decreasing trends were seen in all eight countries with $\sum$ $PBDE_6$ concentrations above 5 ng/g lipid at times of their first participation (Fiji, Barbados, Jamaica, Mexico, Antigua-Barbuda, Haiti, Ireland, and Australia) with a median of about 50% decrease over the years between first and last participation (range 25–80%).

At the global level calculated from these 36 countries, the statistically significant overall decrease rate per 10 years was 32% calculated by the Theil–Sen method and 48% by the median method, respectively (Schächtele et al., 2023b).

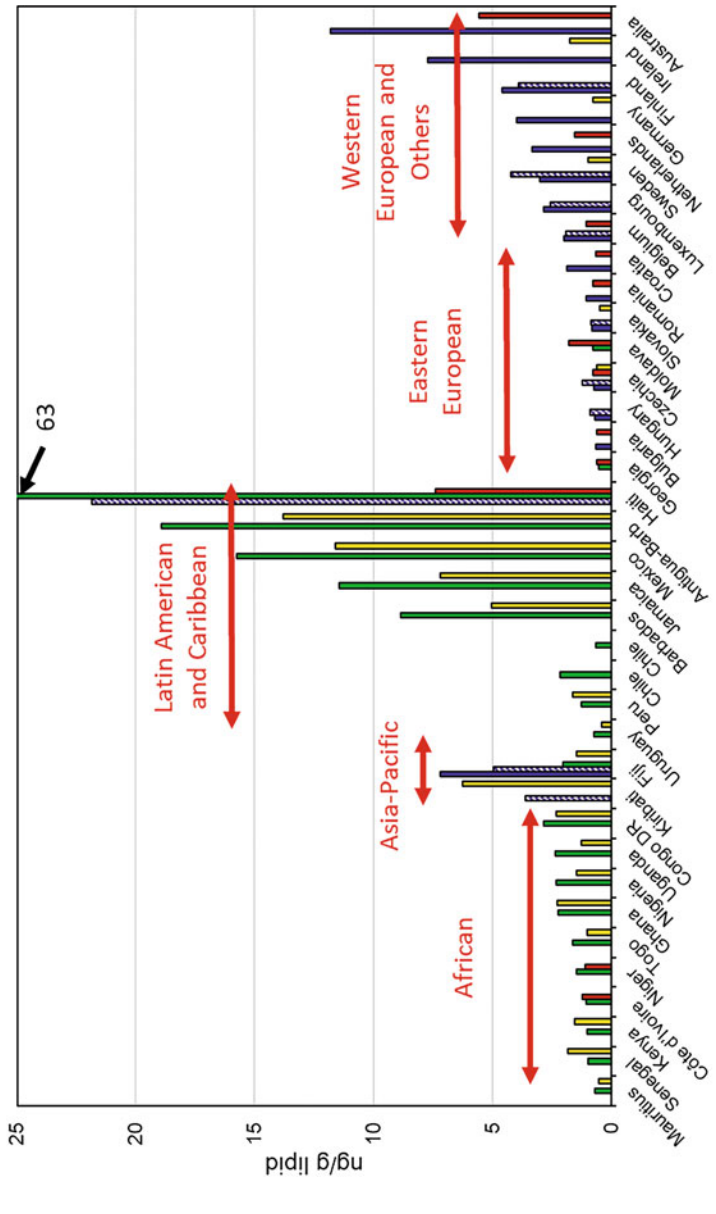

Fig. 33 Overview of the development of $\sum PBDE_6$ concentrations in human milk (ng/g lipid) over time between 2000 and 2019 for 36 countries of the five UN regional groups that participated two or more times

PFOS

As PFAS analysis was initiated with samples submitted in 2008, time trends for PFOS can be derived beginning with the 2008–2011 period. As starting point, descriptive parameter summarizing the results of 86 national pools from 59 countries was used as basis for the first *general* estimation of temporal trends with differentiation into three equal four-year periods between 2008 and 2019. The median of the $\sum$ PFOS concentrations increased from 24.6 ng/L in the 2008–2011 period ($N = 27$) to 36.0 ng/L in the 2012–2015 period ($N = 15$) and then decreased to 13.6 ng/L in the 2016–2019 period ($N = 44$). The highest maximum level (218 ng/L) was found in the 2016–2019 period.

However, the regional distribution of samples and the regional distribution within each of the three periods varied considerably, with most samples originating from the African region. There was no sample from Western European and Others Group in the first period (2008–2011) and no country from this region with repeated participation in these three periods between 2008 and 2019. The second period (2012–2015) had the lowest number of samples in all UN Regions except the Eastern European Group. Note that two countries submitted two samples in different years of the same period: Chile (2008, 2011) and Niue (2017, 2019). The short period between the two samplings in these two countries should not be used for calculation of country-specific temporal trends.

The summarizing descriptive parameters seem to indicate an increase of the PFOS concentrations after listing in 2009 until the 2012–2015 period. Therefore, on basis of countries with repeated participation it was checked whether these fluctuations of the median were likely due to the result of participation of different countries in different rounds of the WHO/UNEP-coordinated exposure studies, as described above for other POPs.

Figure 34 illustrates the temporal tendencies in 24 countries of the four UN regional groups that participated two or more times. In 19 of the 22 countries with repeated participation in different periods, downward tendencies were observed over the years between first and last participation (median decrease: 46%; range 8–78%). In two countries, the PFOS concentrations remained quite constant; in one country, increasing tendencies were seen (Côte d'Ivoire, from 2010 to 2015).

Statistically significant time trends for the UN Regional Groups and worldwide were derived by pooling of two sets of data: (1) using the data from all countries; (2) using the data only from countries with repeated participation (not possible for Asia-Pacific Regional Group and the Western European and Others Group). Overall decrease rates over 10 years between 50% and 80% were achieved in the regions except the Asia-Pacific Group (with an increase of 25% based on the data of all countries) and worldwide computed using all countries or only countries with repeated participation (Table 10).

For details and the temporal trends for PFOA and PFHxS, see Malisch et al. (2023f).

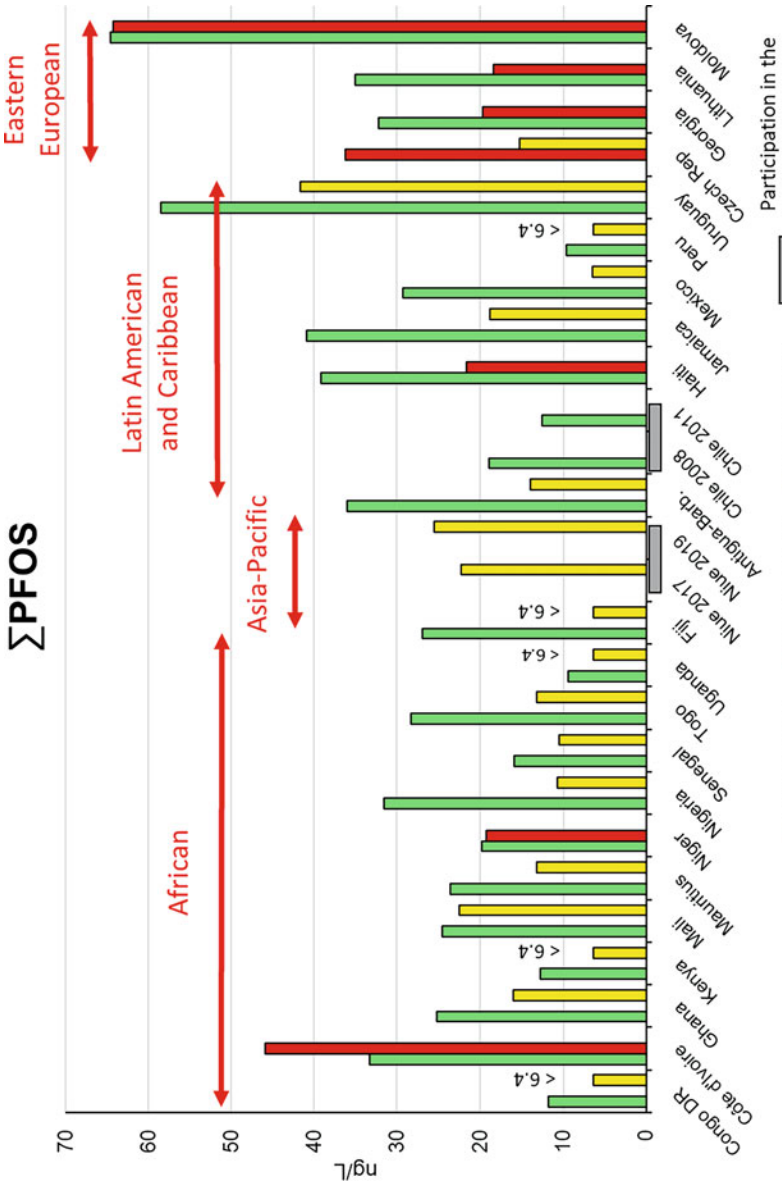

Fig. 34 Overview of the development of ∑ PFOS concentrations in human milk (ng/L) over time between 2008 and 2019 for 24 countries of four UN regional groups that participated two or more times

Table 10 Overall decrease (%) of the ∑ PFOS concentration in human milk (expressed as ng/L) in the five UN Regional Groups and worldwide (n.a. = not applicable) computed (1) using all samples and (2) using samples from countries with repeated participation. Negative decreases are to be read as increase

UN Regional Group	*N* of countries	Overall decrease (%) per 10 years		Trend *p*-value overall
		Theil–Sen method	Median method	
African				
All countries	16	50.3	48.0	<0.001
Countries with repeated participation	11	48.5	48.0	≤0.001
Asia-Pacific				
All countries	16	−24.9	n.a.	0.217
Countries with repeated participation	1	n.a.	n.a.	
Latin American and Caribbean				
All countries	12	69.8	64.2	<0.001
Countries with repeated participation	6	66.0	64.2	0.003
Eastern European				
All countries	8	62.6	64.1	0.002
Countries with repeated participation	4	56.4	64.1	0.052
Western Europe and Others				
All samples	7	79.1	–	0.005
Countries with repeated participation	0	n.a.	n.a.	
Worldwide				
All countries	59	48.3	62.6	<0.001
Countries with repeated participation	24	52.2	52.6	<0.001

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Outlook (Towards Future Studies on Human Milk)

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Abstract

After the comprehensive presentation of the results of the WHO/UNEP studies on human milk in this compendium, this short chapter looks at upcoming needs, challenges, and opportunities. It briefly discusses the candidate chemicals to be reviewed for listing, related challenges for chemical analyses and aligns them with the progress in new techniques and technologies. Further, a need for harmonization of the survey design has also been apparent in recent years, at least at the EU level, in particular in support of the next generation risk assessment and enhanced protection of human health. This fact also represents a new opportunity on how to further develop existing human milk studies to provide necessary management responses.

Keywords

WHO/UNEP-coordinated exposure studies · Human milk · Persistent organic pollutant · POP · Outlook · Future needs · Challenges · Global · Time trend · Relative importance of chemicals · Stockholm Convention on POPs · Emerging contaminants · Exposome · Non-target analysis

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R. Malisch et al. (eds.), *Persistent Organic Pollutants in Human Milk*,
https://doi.org/10.1007/978-3-031-34087-1_17

1 Introduction

As shown in Part I (Introduction) of this compendium, human milk has served over decades as a useful matrix to assess the exposure of the general population to persistent organic pollutants (POPs). The early harmonization and strict parametrization of the former World Health Organization (WHO) field studies allowed to establish a durable framework for monitoring on POPs since the mid-1980s and early 1990s (Fürst 2023). The Stockholm Convention used this framework to monitor POPs in human tissues after defining parameters and core matrices for its Global Monitoring Plan. The range of chemicals increased from the initial focus on polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) in the first two WHO field surveys to 30 chemicals (28 chlorinated or brominated, 2 perfluorinated substances) in the latest, seventh (2016–2019) round of milk surveys (Malisch et al. 2023a). However, there are additional challenges ahead.

2 New Candidate Chemicals, New Requirements

Originally, the POPs were predominantly lipophilic (Fürst 2023), but in recent years the candidate chemicals cover hydrophilic and amphiphilic compounds, such as poly- and perfluorinated candidates that have variable physicochemical properties and require a very different set of techniques to detect these analytes in the samples.

As no multi-method exists allowing to determine all POPs of interest to the Stockholm Convention, various analytical methods have to be applied for the presently listed 30 chemicals as shown in Part II (Analytical methods and quality control) of this compendium. This is even more true for other candidate (POPs) chemicals in the pipeline—already listed was PFHxS in 2022, and the COP will consider listing of dechlorane plus, and sunscreen/UV filter UV-328 and then methoxychlor. Next in the pipeline are chlorpyrifos, chlorinated paraffins with carbon chain lengths in the range C14–17 and chlorination levels at or exceeding 45% chlorine by weight and long-chain perfluorocarboxylic acids, their salts and related compounds (BRS 2022).

Some data on those candidate chemicals in human milk are already available and give us a hint of the magnitude of the problem as well as techniques necessary to detect the new chemicals along the existing set of standard methods. A recent review by (Martín-Carrasco et al. 2023) on several pesticides includes concentrations of methoxychlor and chlorpyrifos in human milk and infant formula from different countries. UV filters, their levels and possible sources in China were studied by (Liu et al. 2022). They report concentrations of 12 UV filters in 100 pooled milk samples from China. The mean 2-(3,5-di-tert-amyl-2-hydroxyphenyl) benzotriazole (UV-328) concentration was 2.6 ± 2.6 ng/g lipid weight. Another survey by (Kim et al. 2019) shows results on UV stabilizers from three Asian countries and finally, (Lee et al. 2015) covers UV-328 levels in human milk collected from Korea with demographic information on the lactating women.

Furthermore, as discussed in the analytical chapter on methods for various POPs including CP (Schächtele et al. 2023), there are challenges in distinguishing between the already listed short chain CP (SCCP) and the candidate chemicals of medium chain CP (MCCP). Indeed, opportunities for using new techniques more broadly are quoted by (Chi et al. 2023) pointing at chemical mixtures and unknown chemicals of potential concern. So far, biomonitoring studies have relied mostly on target analysis. Non-target analysis (NTA) is seen as a tool to improve the characterization of the chemical exposome. HRMS or TOF-MS can identify known and unknown chemicals and are useful tools for non-target screening. "Omics" biomarkers permit the observation and measurement of response modulation at different biological scales. Finally, confirmatory methods allow the unequivocal identification and quantification of POPs present in a sample and provide full information on congener basis.

Last but not least, the expansion of analytes requires continuous amendments of the GMP guidance document (UNEP 2021) to encompass technological progress. This activity regularly takes place by GMP experts once the candidate chemicals complete the assessment procedure and the Conference of the Parties to the Stockholm Convention approves their listing. At that moment experts prepare an overview of available existing scientific knowledge including analytical techniques/methods to analyse newly listed chemicals in core matrices of the Stockholm Convention.

We believe, it may be worth considering another holistic review of the guidance very soon to define further approaches for the long term. Namely, to reconsider type and number of core matrices, corresponding most efficient analytical methods and new techniques for baseline screening and detailed chemical analyses.

3 New Approaches

Building on the two rounds of field studies in human milk coordinated by WHO in the mid-1980s and 1990s on exposure to PCB and PCDD/PCDF, five expanded studies on POPs were performed between 2000 and 2019. To date, the seven rounds of WHO- and/or UNEP-coordinated human milk exposure studies on POPs are the largest global surveys on human tissues with a harmonized protocol spanning over the longest time period and carried out in a uniform format. As shown in Parts III and IV of this compendium, the surveys yielded a comprehensive set of global data covering all targeted POPs listed under the Stockholm Convention in all five UN regions over up to three decades. Therefore, assessments are possible from various perspectives. For the human milk samples of the 2016–2019 period, results for the full set of 32 POPs of interest for the Convention until 2019 (30 listed, 2 proposed for listing as of 2019) are available providing the basis for discussion of the relative importance ("ranking") of the quantitative occurrence of POPs—a unique characteristic among the core matrices under the GMP (Malisch et al. 2023b).

Expanding the list of chemicals of concern (HBM4EU 2016; HBM4EU 2022; WHO 2015) also brings additional regulatory responsibilities/needs. Fast

interventions and promotion of different products, and exposure to mixtures, raise awareness and concerns in public and policy-makers (UNEP 2019). Regulatory decisions on chemicals require more scientific information, including on exposure, as a priority (WHO 2021; EU 2020).

In addition, there have been also other parallel developments related to a broader use of human biomonitoring as a tool contributing to better protection of human health and the environment from negative effects of toxic compounds. The EU has initiated several projects that examined the human biomonitoring (HBM), such as COPHES/DEMOCOPHES in 2009–2012. There are many human biomonitoring surveys and projects in many countries around the world and their number is increasing (Choi et al. 2015). Just in Europe, a total of 192 HBM surveys were reported from 29 European countries in 2017, but not all surveys/projects are using human milk as a matrix (HBM4EU 2017). More recently, the European Initiative for Human Biomonitoring (HBM4EU) in 2016–2021 has aimed at fully harmonizing the accumulated experience (HBM4EU 2022). Last but not least, the EU also declares the importance of human biomonitoring in the EU Chemical Strategy for Sustainability (EU 2020). The same document also emphasizes risk assessment and needs to have a rapid transfer of progress in science directly to decision-making. For implementation of the EU Green Deal and EU Chemicals Strategy, the EU has established a science-to-policy partnership on assessment of risks of chemicals with the aim to develop and endorse new methods for new generation risk assessment (EU 2023).

Further, use of exposure assessments in decision-making gained more importance in recent years, as demonstrated by strengthening regional policy actions, and harmonizing the human biomonitoring surveys has been a chemical safety priority in the Parma and Ostrava Declarations endorsed by Ministers of Health and Environment at the European continent (WHO 2010; WHO 2017). Other examples could be the European Human Exposome Network (EHEN) of nine research projects that addresses health impacts in relation to exposures to air quality, noise, chemicals, and urbanization (EHEN 2022).

What can be the future for such projects and how to define their sustainability? One example of the way forward could be a roadmap for the European Strategy Forum on Research Infrastructures (ESFRI) to develop a pan European network addressing a gap in knowledge on linking environment factors affecting human health—an exposome research (ESFRI 2021). The Environmental Exposure Assessment Research Infrastructure (EIRENE RI) is a network aiming at sustainable research infrastructure enabling the advancement of exposome research in Europe by bringing together complementary capacities available in the member states, harmonizing them and upgrading to address current scientific and societal challenges in the areas of chemical exposures and population health, be it on data mining or further developing monitoring tools and approaches (EIRENE 2022).

4 Needs for the Future

Our ability to assess exposure in the future and derive the effectiveness of adopted measures depends on the availability of comprehensive datasets. We need to adapt to new methods and opportunities, but we also need to maintain the ability to understand and encompass well-established methods—e.g. maintain and regularly repeat the WHO/UNEP human milk survey on POPs, adapting to new POPs of interest that are of potential public health concern, as it has been done over the past decades.

In our view, we see an irreplaceable role for UNEP and WHO coordinated studies performed every four or five years using standard protocols and reliable analytical methods to generate reliable, harmonized, and validated datasets which allow to detect changes in concentrations of target chemicals over time in this core matrix of the Stockholm Convention for evaluation of the effectiveness of policy and management measures.

This includes the need to promote the repeated participation of countries that had already participated in the milk studies as it broadens the dataset to evaluate temporal trends. Furthermore, to increase the number of representatives, other countries should be encouraged to close gaps in the regional/global coverage.

Finally, there is also a need to continue the development of modern technology with (computer based) sustainable tools to support data mining efforts, such as science-policy research partnership PARC activities on data or the development of networks for exposome research such as the EIRENE infrastructure, which would provide open access to interested researchers, but also to other stakeholder communities.

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