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

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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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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

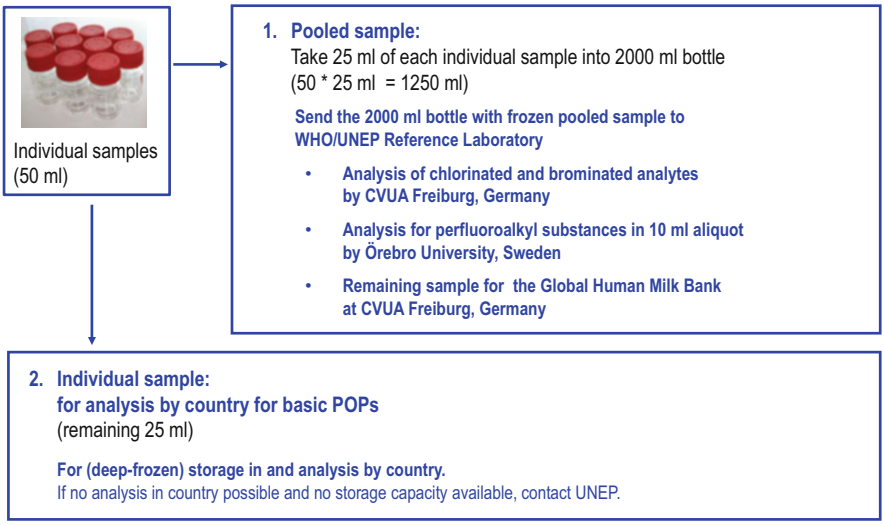

Fig. 1 Concept for individual and pooled samples

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

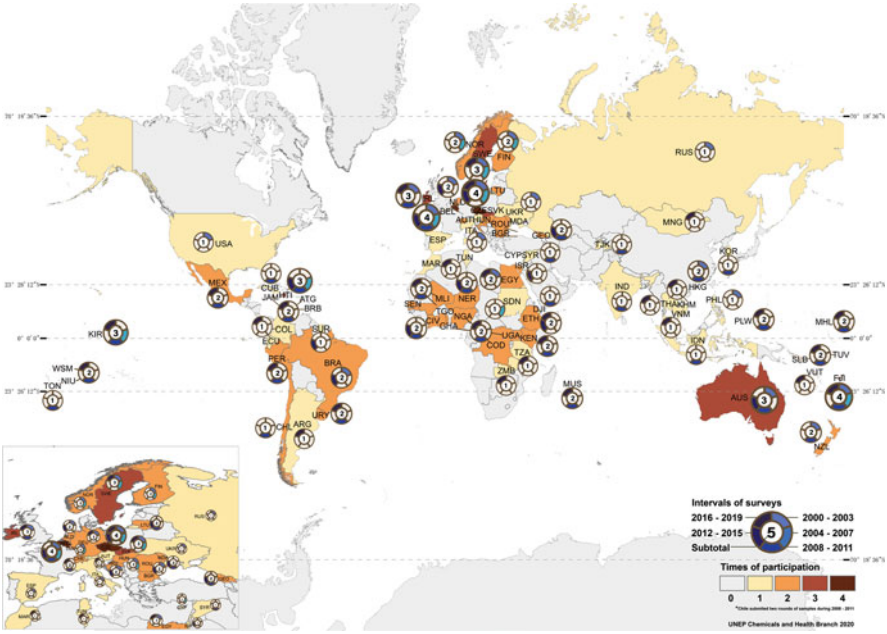

Fig. 2 Regional distribution, number of participations, and time period of participating countries 2000–2019

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.

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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).

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References

European Commission (2006) Commission Regulation (EC) No. 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories, Official Journal of the European Union, L 136, 24.5.2006, p. 3

European Commission (2018) Commission Regulation (EU) 2018/192 of 8 February 2018 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the EU reference laboratories in the field of contaminants in feed and food. Off J Eur Union, L 36/15

Fiedler H, Abad E, van Bavel B, de Boer J, Bogdal C, Malisch R (2013) The need for capacity building and first results for the Stockholm convention global monitoring plan. Trends Anal Chem 46:72–84

Fürst P (2023) Human milk surveys on persistent organic pollutants from a historical perspective. In: Malisch R, Fürst P, Šebková K (eds) Persistent organic pollutants in human milk. Springer, Cham (in this volume, Part I)

Kotz A, Malisch R, Kypke K, Oehme M (2005) PBDE, PBDD/F and mixed chlorinated-brominated PXDD/F in pooled human milk samples from different countries. Organohalogen Compd 67: 1540–1544

Lakind J, Berlin Jr CM (2002) Technical workshop on human milk surveillance and research on environmental chemicals in the United States: an overview. J Toxicol Environ Health A 65: 1829–1838

Malisch R (2001) Stabilization of human milk samples with dichromate. Communication to countries participating in the WHO study

Malisch R, Moy G (2006) Fourth round of WHO-coordinated exposure studies on levels of persistent organic pollutants in human milk. Organohalogen Compd 68:1627–1630

Malisch R, van Leeuwen FXR (2003) Results of the WHO-coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. Organohalogen Compd 64:140–143

Malisch R, Fouzy ASM, Saad MM (2000) PCDD/PCDF in human milk and river Nile fish from Egypt. Organohalogen Compd 48:5–8

Malisch R, Kypke K, van Leeuwen FXR, Moy G, Park S (2008) Evaluation of WHO-coordinated exposure studies on levels of persistent organic pollutants (POPs) in human milk with regard to the global monitoring plan. Organohalogen Compd 70:228–232

Malisch R, Kypke K, Kotz A, Wahl K, Bitomsky N, Moy G, Park S, Tritscher A, Lee SY, Magulova K, Fiedler H (2010) WHO/UNEP-coordinated exposure study (2008-2009) on levels of persistent organic pollutants (POPs) in human milk with regard to the global monitoring plan. Organohalogen Compd 72:1766–1769

Malisch R, Kypke K, Kotz A, Wahl K, Bitomsky N, Moy G, Park S, Tritscher A, Yong Lee S, Magulova K, Fiedler H (2011) Levels of HCH, endosulfan, chlordane, nonachlor, S-421, musk xylene and musk ketone in human milk of WHO/UNEP-coordinated exposure study for determination of POPs (2008-2010) with regard to the global monitoring plan. Organohalogen Compd 73:1023–1025

Malisch R, Schächtele A, van Leeuwen FXR, Moy G, Tritscher A, Šebková K, Klánová J, Kalina J (2023a) Time trends in human milk derived from repeated participation of countries in WHO- and UNEP-coordinated exposure studies, subchapter 1: polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. In: Malisch R, Fürst P, Šebková K (eds) Persistent organic pollutants in human milk. Springer, Cham (in this volume, Part IV)

Malisch R, Hardebusch B, Lippold R, van Leeuwen FXR, Moy G, Tritscher A, Šebková K, Klánová J Kalina J (2023b) Time trends in human milk derived from repeated participation of countries in WHO- and UNEP-coordinated exposure studies, subchapter 2: DDT, beta-HCH and HCB. In: Malisch R, Fürst P, Šebková K (eds) Persistent organic pollutants in human milk. Springer, Cham (in this volume, Part IV)

Malisch R, Fürst P, Šebková K, Klánová J, Sapunova D, Kalina J (2023c) Time trends in human milk derived from WHO- and UNEP-coordinated exposure studies, subchapter 3: perfluorinated alkane substances (PFAS). In: Malisch R, Fürst P, Šebková K (eds) Persistent organic pollutants in human milk. Springer, Cham (in this volume, Part IV)

Ritter L, Solomon KR, Forget J, Stemeroff M, O'Leary C (1998) Persistent organic pollutants—An assessment report on DDT—Aldrin—Dieldrin—Endrin—Chlordane -Heptachlor—Hexachlorobenzene—Mirex—Toxaphene—Polychlorinated Biphenyls—Dioxins and Furans, prepared for the International Programme on Chemical Safety. UNEP, Intergovernmental Negotiating Committee for an internationally legally binding instrument for implementing international action on certain persistent organic pollutants, First session, Montreal, 29 June–3 July 1998, UNEP/POPS/INC.1/INF/10

Schächtele A, Malisch R, Hardebusch B, van Leeuwen R, Moy G, Tritscher A, van Duursen M, van den Berg M, Šebková K, Klánová J, Kalina J (2023) WHO- and UNEP-coordinated human Milk studies 2000–2019: findings of polybrominated substances. In: Malisch R, Fürst P, Šebková K (eds) Persistent organic pollutants in human milk. Springer, Cham (in this volume, Part III)

Schecter A, Pavuk M, Päpke O, Malisch R (2004) The use of potassium dichromate and ethyl alcohol as blood preservatives for analysis of organochlorine contaminants. Chemosphere 57:1–7

Schutz D, Moy GG, Käferstein FK (1998) GEMS/food international dietary survey: infant exposure to certain organochlorine contaminants from breast milk: a risk assessment, World Health Organization document WHO/FSF/FOS/98.4 https://apps.who.int/iris/handle/10665/64097

Šebková K (2023) The Stockholm convention, global monitoring plan and its implementation in regional and global monitoring reports. In: Malisch R, Fürst P, Šebková K (eds) Persistent organic pollutants in human milk. Springer, Cham (in this volume, Part I)

UNEP (2001) Stockholm convention on persistent organic pollutants (POPs), text and annexes. http://www.pops.int

UNEP (2003) Proceedings of a UNEP workshop to develop a global POPs monitoring programme to support the effectiveness evaluation of the Stockholm convention, 24–27 March 2003, Geneva, Switzerland

UNEP (2007a) Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants, Third meeting, Dakar, 30 April–4 May 2007, UNEP/POPS/COP.3/23, 23 February 2007, "Draft implementation plan for the global monitoring plan for the first effectiveness

evaluation", http://www.pops.int/Portals/0/Repository/COP3/UNEP-POPS-COP.3-23.English.pdf

UNEP (2007b) Guidance on the global monitoring plan for persistent organic pollutants, preliminary version, February 2007

UNEP (2007c) Decisions adopted by the Convention of Parties SC-3/19 "Effectiveness evaluation" http://www.pops.int/TheConvention/ConferenceoftheParties/ReportsandDecisions/tabid/208/Default.aspx

UNEP (2009) Report of the conference of the parties of the Stockholm convention on persistent organic pollutants on the work of its fourth meeting, Geneva, 4–8 May 2009, UNEP/POPS/COP.4/38

UNEP (2011a) Conference of the parties to the Stockholm convention on persistent organic pollutants, fifth meeting, Geneva, 25-29 April 2011, regional monitoring reports under the global monitoring plan for effectiveness evaluation: additional human tissue data from the human milk survey. UNEP/POPS/COP.5/INF/28, http://www.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP5/COP5Documents/tabid/1268/Default.aspx

UNEP (2011b) Report of the conference of the parties to the Stockholm convention on persistent organic pollutants on the work of its fifth meeting, Geneva, 25-29 April 2011, UNEP/POPS/COP.5/36, http://www.pops.int/TheConvention/ConferenceoftheParties/ReportsandDecisions/tabid/208/Default.aspx

UNEP (2012) UNEP-coordinated survey of mothers' milk for persistent organic pollutants—guidelines for organization, sampling and analysis. Prepared by Malisch K, and Fiedler H, July 2012, for UNEP Chemicals Branch

UNEP (2013a) Conference of the parties to the Stockholm convention on persistent organic pollutants, sixth meeting, Geneva, 28 April–10 May 2013: results of the global survey on concentrations in human milk of persistent organic pollutants by the United Nations Environment Programme and the World Health Organization. UNEP/POPS/COP.6/INF/33

UNEP (2013b) Persistent organic pollutants in mother's milk in Africa, Latin America and the Pacific Islands. Report prepared by Heidelore Fiedler. ISBN: 978-2-940400-12-7

UNEP (2013c) Report of the conference of the parties to the Stockholm convention on persistent organic pollutants on the work of its sixth meeting, Geneva, 28 April–10 May 2013, UNEP/POPS/COP.6/33, http://www.pops.int/TheConvention/ConferenceoftheParties/ReportsandDecisions/tabid/208/Default.aspx

UNEP (2015) Report of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants on the work of its seventh meeting, Geneva, 4–15 May 2015, UNEP/POPS/COP.7/36, http://www.pops.int/TheConvention/ConferenceoftheParties/ReportsandDecisions/tabid/208/Default.aspx

UNEP (2017a) Conference of the parties to the Stockholm convention on persistent organic pollutants, eighth meeting, Geneva, 24 April–5 May 2017, report on the effectiveness evaluation of the Stockholm convention on persistent organic pollutants, UNEP/POPS/COP.8/INF/40 http://www.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP8/tabid/5309/Default.aspx

UNEP (2017b) Guidelines for organization, sampling and analysis of human milk on persistent organic pollutants. https://www.unep.org/explore-topics/chemicals-waste/what-we-do/persistent-organic-pollutants/guidance-and-standard?_ga=2.268526644.472581330.1659970271-1191406017.1659970271

UNEP (2017c) Report of the conference of the parties to the Stockholm convention on persistent organic pollutants on the work of its eighth meeting, Geneva, 24 April–5 may 2017, UNEP/POPS/COP.8/32, http://www.pops.int/TheConvention/ConferenceoftheParties/ReportsandDecisions/tabid/208/Default.aspx

UNEP (2019a) Guidance on the global monitoring plan for persistent organic pollutants. UNEP/POPS/COP.9/INF/36. http://www.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP9/tabid/7521/Default.aspx

UNEP (2019b) Report of the conference of the parties to the Stockholm convention on persistent organic pollutants on the work of its ninth meeting, Geneva, 29 April–10 May 2019, UNEP/POPS/COP.9/30, http://www.pops.int/TheConvention/ConferenceoftheParties/ReportsandDecisions/tabid/208/Default.aspx

UNEP (2020) All POPs listed in the Stockholm Convention. http://www.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx

UNEP/WHO (2009) Document SC/UNEP/POPS/COP.4/INF/31 "Status report on the human milk survey conducted jointly by the Secretariat of the Stockholm Convention and the World Health Organization", Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants, Fourth meeting, Geneva 4–8 May 2009. http://www.pops.int/TheConvention/ConferenceoftheParties/Meetings/COP4/COP4Documents/tabid/531/Default.aspx

United Nations (2019a) Population division, world population prospects 2019, definition of regions, https://population.un.org/wpp/DefinitionOfRegions/

United Nations (2019b) Statistics division. Methodology—Standard country or area codes for statistical use (M49), https://unstats.un.org/unsd/methodology/m49/

United Nations (2019c) Department for General Assembly and Conference Management (DGACM), United Nations Regional Groups of Member States, https://www.un.org/dgacm/content/regional-groups

van den Berg M, Kypke K, Kotz A, Tritscher A, Lee SY, Magulova K, Fiedler H, Malisch R (2016) WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit–risk evaluation of breastfeeding. Arch Toxicol. https://doi.org/10.1007/s00204-016-1802-z

WHO (1989) Levels of PCBs, PCDDs, and PCDFs in breast milk—results of WHO-coordinated interlaboratory quality control studies and analytical field studies. WHO Regional Office for Europe. Environ Health 34:1–92. ISBN 87-7437-254-8

WHO (1991) Levels of PCBs, PCDDs, and PCDFs in human milk and blood—second round of quality control studies. WHO Regional Office for Europe, Environmental Health in Europe 37: 1–75

WHO (1996) Levels of PCBs, PCDDs and PCDFs in human milk—second round of WHO-coordinated exposure study. WHO European Centre for Environment and Health, Environmental Health in Europe No 3:1–121

WHO (2000a) Interlaboratory quality assessment of levels of PCBs, PCDDs and PCDFs in human milk and blood plasma—fourth round of WHO-coordinated study. WHO European Centre for Environment and Health, Bilthoven, The Netherlands. Processed by WHO Regional Office for Europe, Copenhagen, Denmark, in Health Documentation Services. WHO Report EUR/00/5020352

WHO (2000b) Levels of PCBs, PCDDs and PCDFs in human milk—protocol for third round of exposure studies, WHO European Centre for Environment and Health, March 2000

WHO (2005) Fourth WHO-coordinated survey of human milk for persistent organic pollutants—a protocol for collection, handling and analysis of samples at the country level

WHO (2007) Fourth WHO-coordinated survey of human milk for persistent organic pollutants in cooperation with UNEP—Guidelines for developing a National Protocol. Revised 1 October 2007. http://www.who.int/foodsafety/chem/POPprotocol.pdf

WHO (2019) WHO Regional Offices, https://www.who.int/about/who-we-are/regional-offices