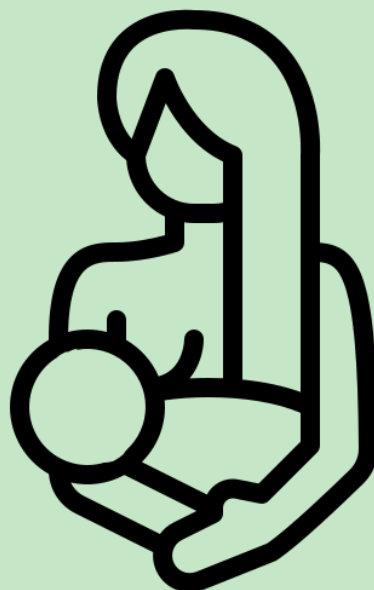


# Sampling of **Persistent Organic Pollutants (POPs) in Human Milk** Methodology and Procedure



April 2026

# ACKNOWLEDGEMENTS

**This document has been prepared by:**

This Standard Operating Procedure (SOP) has been developed under the Global Environment Facility (GEF)-funded programme entitled “Global Chemicals Monitoring Programme to Support Implementation of the Stockholm and Minamata Conventions (GCMP)” (GEF ID 11534), including its global and regional child projects. To support harmonized monitoring of persistent organic pollutants (POPs) and mercury under the GCMP, a series of SOPs has been developed. These SOPs cover the core matrices of air, human milk, and water for POPs, and air for mercury.

The document has been prepared as an updated guidance based on the 2017 UNEP Guidance on Human Milk survey, by the Chemicals Science and Policy Unit, Chemicals and Health Branch, Industry and Economy Division, United Nations Environment Programme (UNEP), in collaboration with Chemisches und Veterinäruntersuchungsamt (CVUA) (State Institute for Chemical and Veterinary Investigation Office (CVUA) Freiburg).

Layout and graphic design: Chemicals Science and Policy Unit.



## ABBREVIATIONS

|       |                                                |
|-------|------------------------------------------------|
| GEF   | Global Environment Facility                    |
| GCMP  | Global Chemicals Monitoring Programme          |
| GMP   | Global Monitoring Plan                         |
| HDPE  | High-density polyethylene                      |
| ID    | Identification                                 |
| ISO   | International Organization for Standardization |
| PFASs | Per-and polyfluoroalkyl substances             |
| POPs  | Persistent Organic Pollutants                  |
| SOP   | Standard Operating Procedure                   |
| UNEP  | United Nations Environment Programme           |
| WHO   | World Health Organization                      |

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# 1. INTRODUCTION

Since 2007, the global monitoring plan (GMP) of persistent organic pollutants (POPs) has been established to provide comparable monitoring data on the presence of POPs listed in Annexes A, B, and C of the Stockholm Convention and to track changes over time. Among the core matrices used to evaluate changes in POPs concentrations over time, human milk and human blood were selected to assess human exposure. The biomonitoring component of the GMP has been established through the joint implementation by the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) (UNEP 2021). Monitoring of human milk allows countries and regions to identify contamination problems affecting the most vulnerable population groups i.e. women of childbearing age and their offspring. It further allows formulating measures to reduce and prevent human and environmental exposure to POPs. Building on the WHO's previous human milk monitoring studies, UNEP and the WHO jointly initiated a global survey to monitor changes in human exposure over time (UNEP 2023). **The survey is implemented worldwide covering countries with large differences in food consumption patterns and environmental levels of POPs.**

Since 2005, UNEP has implemented POPs monitoring projects through Global Environment Facility (GEF) funded GMP initiatives, strengthening POPs monitoring capacities in developing and transitioning countries. These projects have contributed data to the effectiveness evaluation of the Stockholm Convention.

**This standard operating procedure (SOP) provides support to the Global Chemicals Monitoring Programme (GCMP) and its child projects in different UN regions by outlining procedures for conducting surveys on the biomonitoring of POPs in human milk.** The GCMP includes six child projects with participating countries spanning across Africa, Asia and Pacific Islands, and Latin America and the Caribbean, with financial support from the GEF. The programme aims to provide scientifically robust evidence for evaluating the effectiveness of the Stockholm Convention on POPs and the Minamata Convention on Mercury. Its overarching goal is to facilitate a comprehensive global understanding of the concentrations of POPs and mercury in humans and the environment.

**The objective of this guidance document is to provide a systematic framework for all activities and tasks associated with the organization, sampling and analysis of human milk samples for the biomonitoring of POPs.** Experiences from previous human exposure studies coordinated by UNEP and WHO (Malisch *et al.* 2023) were used and updated where necessary during the preparation of this guidance document. Notably, the 2007 guidelines of WHO for developing a National Protocol (WHO 2007) supporting the global human milk

studies were amended to take into account various practical considerations and also to accommodate the guidelines for newly listed POPs. The present guidance document represents an update of the previous guidelines “UNEP-coordinated Survey of Human Milk for Persistent Organic Pollutants Guidelines for Organization, Sampling and Analysis” (UNEP 2017).

## 1.1. Health benefits of breastfeeding

**Breastfeeding provides the most crucial nutrients for the infant’s health, providing perfect nutrition and antibodies that protect against infections, allergies, and lifelong chronic diseases including obesity.** No effort by governments, the WHO, the United Nations Children’s Fund (UNICEF), and other international organizations should be spared to promote exclusive breastfeeding up to six months of age, with continued breastfeeding up to two years. All persons involved in this survey, and especially those who have direct contact with potential study participants, should be well informed about the health benefits of breastfeeding for the infant as well as the mother (see [Annex 1](#)).

While national protocols on human milk sampling may require flexibility, the following general principles should be observed:

- Breastfeeding should be protected, promoted, and supported.
- The health benefits of breastfeeding to both the mother and the infant should be clearly and consistently communicated.
- Sampling of milk should not be an undue burden on the mother, nor should it compromise the nutritional status of the infant.
- Human milk sampling should be carried out in an environment with safe and comfortable space for both the mother and the infant.
- Ethical review, including prior informed consent, should be respected.
- Safeguarding of confidential information should be assured.
- Quality assurance of results should be independently confirmed.

## 2. IMPLEMENTATION ASPECTS OF HUMAN MILK SURVEYS

The GCMP includes five UN Regions and sub-regions, including Africa, Asia and Pacific Islands, and Latin America and the Caribbean Islands.

**For each country participating in the GCMP regional project, a National Coordinator needs to be identified who will be responsible for the overall planning and implementation of the survey in the country, and will be assisted by respective health, laboratory, and administrative staff.** In particular, the National Coordinator should assure that the survey meets all national ethical requirements for human biomonitoring. The Guidelines for Developing National Protocols by the WHO (WHO 2007) offer guidance for constructing national protocols to monitor POPs in human milk. These guidelines were developed based on the advice and recommendations of the ad-hoc WHO Human Milk Survey Advisory Group. Additionally, during the previous UNEP/GEF GMP2 projects, participating countries prepared national protocols that were approved by their respective national ethics committees. These protocols, along with the current SOP, can serve as a foundation for developing new protocols.

The continuous exchange of information between the regional centre in charge of executing the GCMP's child projects, UNEP, the designated expert and/or regional laboratory and the national coordinators on basic information such as nominations and contact details are a prerequisite for the successful organization of a study at regional and global levels. The regional centres, UNEP and the participating laboratories should maintain a permanently updated list of participants and contact points and continuously exchange relevant information. **The national coordinators should establish close contact with the regional centre, UNEP, and the laboratories for the exchange of information on the status of sample collection, in particular with regard to the coordination of shipments.**

### 2.1. Concept of the survey

**The following considerations form the basis of the general outline of the survey:**

For the purpose of Article 16 of the Stockholm Convention, the need to quantify concentrations of POPs over time makes it essential to identify comparable cohorts so that, in subsequent years, statistically reliable evaluations of time trends can be performed. In certain countries, it may be of interest to collect individual samples from different regions. In addition, variations in dietary patterns or locally contaminated areas may be of interest. Although these aspects may not be directly related to Article 16 of the Stockholm Convention, countries may wish to incorporate some of them into their protocols, provided that the main

aims of the survey are not compromised.

## 2.2. General idea of collection of individual samples, preparation of pooled samples and role of expert laboratories

**To ensure statistically reliable data, each country is advised to collect at least 50 individual samples, which are then pooled to form a representative composite sample.** Countries with population over 50 million should include one additional donor per million inhabitants above 50 million. For countries with substantially large populations and sufficient resources, the collection of multiple pooled samples is encouraged. [Chapter 3](#) provides detailed information on the appropriate number of individual samples and the selection of donors.

This pooling approach is more cost-effective and requires smaller milk volume per donor as compared to analysing individual samples. It also allows for a more comprehensive analysis at lower quantification limits.

By performing the analysis of pooled samples (being representative of the participating countries) in a single expert laboratory, a high degree of reliability of the analytical results can be achieved. Such data are essential for the statistical validation of changes in POP concentrations over time. Therefore, the use of representative pooled samples, with the determination of all analytes of interest in an expert laboratory, has continuously been a key element in monitoring concentrations of the relevant POPs in human milk.

**Furthermore, the SOP allows countries to stratify participants to reflect presumed national exposure profiles.** Factors to consider when selecting study participants include diet, agricultural practices, occupational exposures, rural vs. urban residence, and proximity to potential POPs sources (e.g., waste sites or industrial activities). This stratification should remain consistent across monitoring rounds to enable the tracking of changes and trends. Since exposure profiles are not well defined in most developing countries, assumptions may be necessary but should be documented as part of the information package.

**For countries with sufficient resources, the SOP allows submission of two pooled samples consisting of different sets of participants.** Although the protocol targets individual countries, stratification and sample collection may be organized regionally. Nevertheless, efforts should prioritize participation from as many countries and regions as possible to establish a comprehensive baseline (UNEP 2021).

## 2.3. Analytes of interest for determination in nationally representative pooled and individual human milk samples

**Table 1** presents the POPs listed under the Stockholm Convention of 2025 (parent compounds and their relevant isomers/congeners/salts). These are to be determined in the representative pooled national human milk samples by the expert laboratory, in accordance with the GMP guidance (UNEP 2021).

**Table 1.** POPs listed under the Stockholm Convention (parent compounds and relevant transformation products) to be determined by the expert laboratory in the representative pooled national human milk samples.

| COP               | POPs or POPs group                                                             | Recommended analytes                                                                                                                                         |
|-------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Initial POPs 2001 | Aldrin                                                                         | Aldrin                                                                                                                                                       |
|                   | Chlordane                                                                      | cis- and trans-chlordane, cis- and trans-nonachlor, and oxychlordane                                                                                         |
|                   | Dichlorodiphenyltrichloroethane (DDT)                                          | 4,4'-DDT, 2,4'-DDT, 4,4'-DDE, 2,4'-DDE, 4,4'-DDD, and 2,4'-DDD                                                                                               |
|                   | Dieldrin                                                                       | Dieldrin                                                                                                                                                     |
|                   | Endrin                                                                         | Endrin                                                                                                                                                       |
|                   | Hexachlorobenzene                                                              | HCB                                                                                                                                                          |
|                   | Heptachlor                                                                     | Heptachlor and heptachlor epoxide                                                                                                                            |
|                   | Mirex                                                                          | Mirex                                                                                                                                                        |
|                   | PCB – indicators PCB and dioxin-like PCB (dl-PCB)                              | ΣPCB <sup>6</sup> (six congeners): 28, 52, 101, 138, 153, and 180<br>dl-PCB (twelve congeners): 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 |
|                   | Polychlorinated dibenzodioxins (PCDDs)                                         | 2,3,7,8-substituted PCDD (seven congeners)                                                                                                                   |
|                   | Polychlorinated dibenzofurans (PCDFs)                                          | 2,3,7,8-substituted PCDF (ten congeners)                                                                                                                     |
|                   | Toxaphene                                                                      | Congeners P26, P50, P62                                                                                                                                      |
| COP-4 2009        | Chlordecone                                                                    | Chlordecone                                                                                                                                                  |
|                   | alpha-hexachlorocyclohexane                                                    | α-HCH                                                                                                                                                        |
|                   | beta-hexachlorocyclohexane                                                     | β-HCH                                                                                                                                                        |
|                   | Lindane                                                                        | γ-HCH                                                                                                                                                        |
|                   | Hexabromobiphenyl                                                              | PBB 153                                                                                                                                                      |
|                   | Pentachlorobenzene                                                             | PeCBz                                                                                                                                                        |
|                   | Tetra- and pentabromodiphenyl ether                                            | PBDE 47, 99, 153, 154, and 175/183 (co-eluting),                                                                                                             |
|                   | Hexa- and heptabromodiphenyl ether                                             | Optional: PBDE 17, 28, and 100                                                                                                                               |
|                   | Perfluorooctanesulfonic acid                                                   | PFOS (linear and branched PFOS, and SPFOs) for air, precursor compounds: FOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE                                        |
| COP-5 2011        | Endosulfan                                                                     | α-, β-endosulfan, and endosulfan sulfate                                                                                                                     |
| COP-6 2013        | Hexabromocyclododecane                                                         | α-HBCD, β-HBCD, γ-HBCD                                                                                                                                       |
| COP-7 2015        | Polychlorinated naphthalenes                                                   | PCN                                                                                                                                                          |
|                   | Hexachlorobutadiene                                                            | HCBD                                                                                                                                                         |
|                   | Pentachlorophenol                                                              | PCP, and PCA                                                                                                                                                 |
| COP-8 2017        | short-chain chlorinated paraffins (C <sup>10</sup> - C <sup>13</sup> ) alkanes | SCCPs                                                                                                                                                        |
|                   | Decabromodiphenyl ether                                                        | PBDE-209                                                                                                                                                     |
| COP-9 2019        | Dicofol                                                                        | Dicofol                                                                                                                                                      |
|                   | Perfluorooctanoic acid                                                         | PFOA                                                                                                                                                         |
| COP-10 2022       | Perfluorohexanesulfonic acid                                                   | PFHxS                                                                                                                                                        |
| COP-11 2023       | Dechlorane plus                                                                | Dechlorane plus                                                                                                                                              |
|                   | Methoxychlor                                                                   | Methoxychlor                                                                                                                                                 |
|                   | UV-328                                                                         | UV-328                                                                                                                                                       |
| COP-12 2025       | Medium-chain chlorinated paraffins (MCCPs; C <sup>14</sup> - C <sup>17</sup> ) | MCCPs                                                                                                                                                        |
|                   | Long-chain perfluorocarboxylic acids (LC-PFCAs)                                | LC-PFCAS                                                                                                                                                     |
|                   | Chlorpyrifos                                                                   | Chlorpyrifos                                                                                                                                                 |

## 3. DEVELOPING A NATIONAL PROTOCOL

These SOP guidelines are intended to assist the National Coordinator in each country in developing a national protocol that is practical, feasible, and sustainable, and that meets the aims of the survey, especially generating comparable monitoring data over time.

In order to obtain statistically reliable data, an appropriate number of individual donors must be recruited to provide samples for the survey. As a first approximation, a minimum of 50 individual samples is recommended for each country. Information on the number of infants born to primipara (first-time) mothers should be available from the health statistics office in the country.

**However, it is recognized that some flexibility in recruiting individual donors may be necessary for countries with small populations and/or low birth rates.** If this is the issue, extending the sample collection period should be considered as the first option to reach a statistically sufficient number of donors. In some cases, reducing the number of donors may be unavoidable, but the impact on the statistical power of the survey to detect differences between time periods should be carefully considered.

**On the other hand, the power of the survey can be increased by including more than 50 individual samples, and this is encouraged.** In particular, countries with populations greater than 50 million should include at least one additional donor per million inhabitants above 50 million. Countries with populations well over 50 million (or with sufficient resources) are encouraged to prepare a second pooled sample (or more) if feasible. It is the responsibility of the National Coordinator to ensure that the number of samples collected for analysis provides a sufficient statistical basis to allow scientifically valid assessments of changes in POPs concentrations over time.

### 3.1. Selection of donors

The national survey protocol should be developed to assess the concentrations of POPs and their changes over time in a defined cohort within the country. **In many countries, collection of samples will be conducted at health clinics providing postnatal services. Therefore, the selection of clinics may be as important as the selection of donors, particularly with regard to staff and facilities.**

As the human exposure to POPs occurs mainly through food, dietary patterns and the concentrations of POPs in those food items primarily determine the concentrations of POPs in

human milk. The location of residence, usually urban or rural, may also be associated with different exposure levels to certain POPs. Living in highly polluted areas, such as in the vicinity of incinerators; textile, upholstery, leather, apparel, and carpets industries; pulp and paper industries; and metal industries, or in areas where organochlorine substances are produced or used, is also known to influence exposure to POPs. **Persons with markedly different exposure to POPs, including occupational exposure, should not be included in the survey to avoid skewing the results.**

**Box 1.** *Important criteria for recruiting donors in the study*

- **Mother should be primipara** (i.e. women giving birth for the first time)

Other recommended criteria are:

- Mother should be under 30 years of age.
- The National Coordinator may consult the National Health Statistics (or equivalent) office for guidance on setting the maximum age to ensure a sufficient number of potential donors. In order to further reduce variability, specifying an age range may be considered a useful criterion.
- Both mother and child should be apparently healthy, including normal pregnancy.
- Mother should be breastfeeding one child only (i.e., mother of a singleton).
- Mother should have resided in the represented area (and country) for at least the last ten years.
- Mother should not reside or work in areas where direct emissions of POPs are known or suspected to result in elevated concentrations of POPs in the local population.
- Mother should be available for sample collection within 3 to 8 weeks of delivery.

Given the differences in pre- and postnatal care in countries, two general procedures to identify possible donors exist. These procedures are described below:

**a. Selection prior to delivery:** In countries with adequate prenatal coverage, possible donors can be contacted before giving birth. All potential donors should be informed about the benefits of breastfeeding and encouraged to breastfeed, even if they do not intend to participate or are not selected in the survey. Once a participant indicates willingness to take part in the survey, she should be invited to complete Sections 1-3 of the questionnaire (see [Annex 6](#)). In addition, the informed consent form might also be completed at this stage. The questionnaire may be completed through a personal interview at the prenatal clinic or by the potential donor at home and returned to the clinic, either in person or by mail. The National Coordinators should determine the most appropriate means of collecting this information. Depending on the homogeneity of the population, up to 250 completed questionnaires should be collected and sent to the National Coordinator for screening and final selection of survey donors. Selected donors should be notified of their inclusion in the survey and of the time and location of sample collection. For most countries, 50 potential donors should be selected. In addition, 10 reserve donors should be identified in case some selected donors become unavailable. The criteria for selecting participants are discussed above in this box. Note that individuals who are not selected to participate in the survey

should be informed and thanked for their time and interest.

**b. Selection post delivery:** It is also possible to recruit donors after the child-birth, i.e. without pre-selection as above. This can be done at postnatal clinics and other relevant venues, i.e. well-baby clinics. Mothers should be interviewed, and Section 2 of the questionnaire should be completed (see [Annex 6](#)). If eligible, Sections 3-4 of the questionnaire should be completed, and the donor should sign the informed consent form. Samples can then be collected, either immediately or later at home. While this method can reduce the duration of the survey by up to four months, it does not allow for further stratification of the cohort to reduce variability. However, once cohort selection criteria have been established from the first sample collection, this approach offers advantages for the second and subsequent sample collections.

**Each procedure has benefits and disadvantages, but the second (i.e. b. Selection post delivery) is the simplest and most efficient.** Furthermore, this avoids considerable delay in the project that would otherwise be caused by the time and efforts required to obtain 250 completed questionnaires from donors contacted before giving birth, in order to select 50 potential donors after giving birth. Therefore, given the tight time frame, the selection of donors after giving birth is recommendable for these projects.

## 3.2. Interviewing potential donors

**The model questionnaire for donors (see [Annex 6](#)) should be used as the basis for recording information regarding their participation in the study.** Note that the National Coordinator should carefully review the questionnaire to ensure applicability to the country. Special consideration should be given to the exclusion criteria. If necessary, these criteria should be modified to balance the need for a suitable pool of possible donors with the aim of reducing factors that contribute to variability.

### 3.2.1. Interviewing potential donors prior to delivery

**For pre-delivery selection, initial interviews should be conducted at prenatal clinics at least two months prior to delivery.** Interviewers should be informed on benefits of breastfeeding (WHO and UNICEF 2020) as well as available local support services through health services or in the community. The interviewer should first ascertain whether the potential donor plans to breastfeed her infant. For potential donors who have not yet decided, this may be an opportunity to provide information on the benefits of breastfeeding for both mother and infant. The discussion should focus on real or perceived obstacles to breastfeeding and how these may be overcome. In certain cases, pregnant women may be referred for breastfeeding counselling if this is deemed useful. If the potential donor does not intend to breastfeed, the interview should be terminated.

**The number of prospective donor mothers interviewed should be large enough to identify an adequate number of qualified participants.** Note that the National Coordinator should be aware of potential sources of POPs and other pollution hot spots and donors living near these locations should be excluded, unless a special cohort is being recruited. The National POPs Contact Point should be consulted, as relevant information may be available in the National Implementation Plan (see the [Stockholm convention Secretariat website for a list of National POPs Contact Points](#)). Completed questionnaires should be sent to the National Coordinator for the final selection of donors. As the information in questionnaires

is confidential, these should be handled with care and according to applicable national data protection requirements. Based on responses to Sections 1-3 of the questionnaire, the National Coordinator should develop selection criteria that promote the most comparable and reliable survey results. In order to assess changes in exposure levels over time, it is very important that the criteria for selecting donor mothers are sufficiently robust to be reproducible in future surveys.

**National Coordinators should review the questionnaires of potential donors and select 50 donors that best meet the criteria for inclusion in the survey.** In addition, reserve donors (i.e., 10 individuals) should be identified in the case some selected donors become unavailable or otherwise withdraw from the survey. Selected donors should be notified of their inclusion in the survey and invited to provide a sample of their milk 3 to 8 weeks after the delivery at a designated postnatal clinic.

### 3.2.2. Interviewing potential donors post delivery

**Alternatively, donors may be selected post delivery, provided that they meet the basic criteria described in Section 2 of the questionnaire.** Once the criteria have been established, this approach is perhaps the simplest, most cost-effective and most time-efficient method for identifying donors.

### 3.2.3. Questionnaire for collecting relevant information from donors and controlling of eligibility criteria

If the donor fulfils the criteria and indicates her willingness to primarily or exclusively breast-feed, the interviewer should generally explain the background and purpose of the survey as described in the summary information (see [Annex 2](#)). A copy of this information should be also provided to the donor in the local language.

If the donor indicates an interest in participating in the survey, Sections 1-2 of the questionnaire (see [Annex 6](#)) should be completed. In administering the questionnaire, if the answer to any of the questions in Section 2 is “no” (with the exception of question 7), the person cannot participate in the survey. Note that if a donor is disqualified due to her age and/or her residence time in the area, her actual age and/or residence time should be recorded. This information may not otherwise be available and may be used in the future to revise selection criteria. However, if answers to Section 2 are all “yes”, except for question 7, which should be “no”, Section 3 should be completed. Note that Section 4 should be completed at the time of sampling, along with the informed consent form, if required.

Those completing the questionnaire may be offered a small gift to compensate for their time. This item should be of nominal value and ideally promote breastfeeding by the mother - for example, a small pillow for supporting the baby during breastfeeding.

### 3.2.4. Handling of filled questionnaires and submission of summary information

**The National Coordinator should retain questionnaires of all respondents until the end of the study. However, questionnaires of finally selected donors should be retained for future reference.** Retention of all records should conform to national requirements and internation-

al norms concerning confidentiality and data protection. The National Coordinator should complete a **summary information form** on mothers donating samples for the pooled sample and submit it to UNEP (see [Annex 5](#)).

### 3.3. Suitable containers for collecting and storing milk samples and preparing pooled samples

The recommended containers for collection of samples are high density polyethylene bottles (HDPE) with HDPE screw caps (see [Figure 1](#)). The use of HDPE containers reduces the risk of breaking of containers during handling and shipment considerably.



**Figure 1.** HDPE containers for human milk sampling (typically 60 bottles of 125 ml, and 1 bottle of 2000 ml per country)

**Before shipment the containers are washed with distilled water and subsequently cleaned with ethanol and/or ethyl acetate.** After this pre-cleaning process the containers are ready to ship and use.

Within the framework of the present programme, each regional executing agency, in coordination with the Steering Committee and the Scientific Advisory Committee, will decide on the best supplier of sampling materials (i.e. containers) for the collection and storage of individual breast milk samples, and will receive shipments of the pooled breast milk sample from the participating country for POPs analysis. **In order to avoid possible contamination**

and to ensure globally harmonized collection of samples, it is proposed that a centralized laboratory with wide experience be in charge of providing and preparing the sampling material for each participating countries.

For customs control at country border, the provider of sampling materials must attach a pro-forma invoice declaring an appropriate value of the shipment in order to avoid the payment of custom fees.

### 3.4. Collection of human milk samples

**Breast milk sampling can be carried out between three to eight weeks (from 21 days to two months) post delivery.** At the time of sample collection, individual interviews should be conducted to complete the participant questionnaire (see [Annex 6](#)). Donors should have already received both verbal and written information regarding the survey. The survey procedures should be explained, particularly the donor's right to withdraw from the survey without prejudice. Following this, donors should be requested to provide their written consent using a standard "**Informed Consent Form**" ([Annex 4](#)). The sample can then be collected.

Donors should provide the sample at a local contact place where collection can be supervised. **At least 50 ml of breast milk in total should be collected** by manual expression after a feeding or while infant is nursing on the other breast, in order to take advantage of the let-down reflex. If the donor prefers, the sample may be also collected at home, in which case manual expression is preferred. In such cases, she should be given detailed instructions for collecting, storing, and transporting of milk samples (see [Annex 3](#)). The person providing the instructions should check the donor's understanding of the procedure. Donors should also be given a clean HDPE bottle with a screw cap to collect and store the milk sample. Sample collection bottles should be labelled with the donor's individual identification code rather than just the donor's name.

### 3.5. Preservation of samples after collection

#### 3.5.1. By freezing

**The sample should be collected directly into the clean HDPE bottles provided.** If the sample is collected at home, it should be collected in HDPE bottles and stored in freezer until it can be delivered to the survey team. In general, milk samples should be stored in a refrigerator at about 4 °C for a maximum of 72 hours, or for longer periods in a freezer at -20°C.

In countries where temperature control is not possible, countries should contact the expert laboratory and UNEP for advice on the best options for preserving samples.

### 3.6. Biosafety

**One of the criteria for selecting donors is that both the mother and the infant should be apparently healthy, with a normal pregnancy.** The reasons for this criterion are to avoid extra demands on a potential donor who is already experiencing difficulties and to minimize variability in results that may be caused by medical conditions (for example, sudden loss of weight may alter the body burden of POPs and their concentrations in human milk).

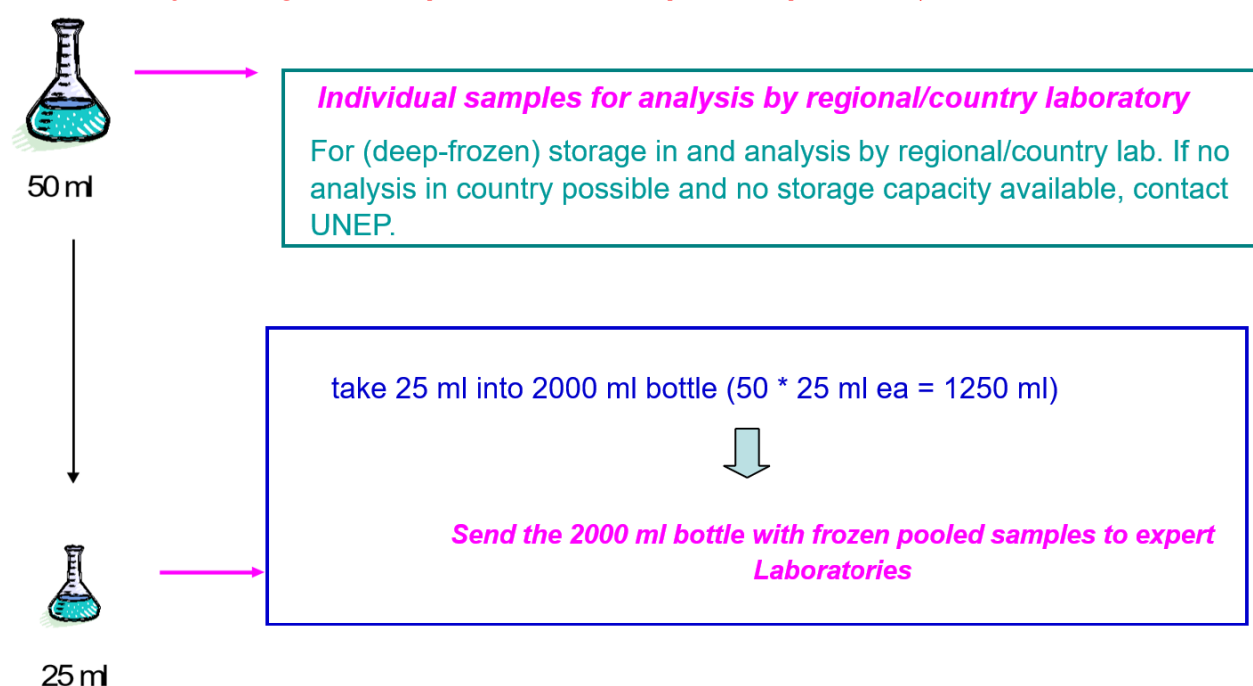
Consequently, donors with previously diagnosed clinical hepatitis, malaria, AIDS, and other such diseases should be excluded from the survey. In many countries, pregnant women are screened for a number of infectious diseases, allowing their health status to be evaluated.

**In countries where HIV screening of pregnant women is established, the National Coordinator should decide whether HIV-positive donors should be excluded from the study.** In this regard, potential weight loss of such donors could be an issue, as well as the biosafety of the samples. In some countries, discrimination based on HIV status is not allowed legally, and in certain countries, a person's HIV status is considered confidential. Although the infectivity of human milk from HIV-positive mothers is considered low when ingested by infants, for the purpose of this study, such milk should be considered infectious unless it has been decontaminated. Therefore, any milk sample known or suspected to be contaminated with HIV should be decontaminated by heating at 62.5 °C for 30 minutes. **Similarly, in countries with a high HIV morbidity and no HIV screening, human milk samples should be considered potentially contaminated and heat-treated as above.**

### 3.7. Preparation of pooled samples

**Prior to preparing pooled samples, the 50 individual milk samples are homogenized by shaking for 5 minutes.** Subsequently, each 50 ml individual samples should be divided into two portions of 25 ml each (see Figure 2). One 25 ml portion is retained as an individual sample and can be analysed by the regional or national laboratory for analytically simple POPs (if capacity exist), while the other 25 ml portion is transferred into a 2000 ml bottle to prepare the pooled sample ( $50 \times 25 \text{ ml} = 1250 \text{ ml}$  pooled sample). In this way, it is ensured that all pooled samples provide the minimum required amount of 500 ml for analyses and 750 ml for the WHO/UNEP Global Human Milk Bank. Each individual and pooled sample is to be labelled with a unique identification code (see Section 3.2.4).

Sample preparation scheme: Preparation of individual and pooled (mixed) samples  
**(Before taking an aliquot, shake thoroughly at room temperature and then take the aliquot immediately. Storage and shipment of all samples deep-frozen.)**



**Figure 2.** Sample preparation scheme

### 3.8. Transporting of samples and contact information of the expert laboratory

The individual samples containing 25 ml each should be sent to the designated national/regional laboratory and the pooled sample to the expert laboratory accompanied by the completed summary of information (see [Annex 5](#)).

Once the samples are shipped, the receiving laboratory shall be notified about the dispatch date, shipment tracking details, and its expected time of arrival. In particular, before the long-distance shipment of samples to the expert laboratory, the National Coordinator is strongly advised to contact the expert laboratory.

**The contact e-mail of CVUA Freiburg (expert laboratory) is [pops@cvuafr.bwl.de](mailto:pops@cvuafr.bwl.de)**

The contact person at CVUA Freiburg for any organizational questions is **Dr. Alexander Schaechtele** ([alexander.schaechtele@cvuafr.bwl.de](mailto:alexander.schaechtele@cvuafr.bwl.de))

**The frozen samples should be sent to the following address:**

Dr. Alexander Schaechtele  
Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg  
(State Institute for Chemical Analysis and Veterinary Diagnostics Freiburg)  
Bissierstr. 5  
D-79114 Freiburg  
Germany

Shipping of the samples shall be carried out by commercial express carriers or other means, in the most expeditious manner possible. Samples should be frozen at  $-20^{\circ}\text{C}$ , packed with cooling elements, and sent to the destination.

**NOTE:** Countries should not use dry ice, as it is classified as a hazardous good (i.e. for DHL and TNT in Germany) and will be only transported if these couriers have a specific contract with the sender. In countries where temperature control is not possible, they should contact the expert laboratory, the regional centre, or UNEP for advice on the best option for preserving the samples.

**With each shipment, a customs declaration must be attached that will be provided on request.** In this custom declaration it is stated that:

1. The human breast milk samples are not infectious.
2. The human breast milk samples do not have a commercial value.
3. The human breast milk samples are shipped to the expert laboratory in Germany, only for analytical reasons.
4. The human breast milk samples will not be used for any kind of application for humans or animals or for any kind of pharmaceutical reasons.

This declaration must be attached to the shipment. Upon arrival of the pooled human milk samples, the bottles should be stored at  $-20^{\circ}\text{C}$  in a freezer. The national or expert laboratory should confirm receipt of the package to sender and UNEP.

## 4. ANALYSIS OF POPs IN HUMAN MILK SAMPLES

### 4.1. Analysis of individual samples

**The 50 individual samples can be analysed for POPs at the national/regional laboratories.** A number of analytical methods using gas chromatography with electron capture detector are available, e.g., AOAC and EPA. The method chosen should preferably have limits of determination low enough to quantify the concentrations anticipated to be present in the samples. The fat content of the milk should be extracted and analysed and results should be reported on a fat basis. In this regard, the recent literature should be consulted.

Emphasis in the identification of the regional/national laboratory should be placed on analytical proficiency, as demonstrated by adequate quality assurance procedures and confirmed through successful participation in the inter-laboratory studies. Based on past analytical quality assurance studies, some laboratories have had difficulty in qualifying for POPs proficiency. Note that this has been observed in laboratories in both developing and developed countries.

Note that mean results of individual analysis can be compared with the result of the pooled sample analysed by the expert Laboratory.

### 4.2. Analysis of pooled samples

**In the previous two round of UNEP/GEF monitoring projects a centralized expert laboratory was appointed for the analysis of the pooled human milk samples,** the State Institute for Chemical Analysis and Veterinary Diagnostics (CVUA) in Freiburg, Germany, for the POPs listed in the Stockholm Convention (parent compounds and relevant transformation products) (see [Table 1](#)). This laboratory has the infrastructure and the experience for the analysis of practically all the POPs listed in Annexes A, B, and C of the Stockholm Convention.

This laboratory has met all the requirements established by the WHO at the Fourth Round for the Interlaboratory Calibration of PCDD, PCDF, and PCB levels in human milk, and has been selected as the EU Reference Laboratory for halogenated POPs in Feed and Food, and as the EU Reference Laboratory for Pesticides in Food of Animal Origin and Commodities with High Fat Content.

This expert laboratory follows a rigorous quality control program to ensure the accuracy and reliability of the chemical analysis and its results. The laboratory routinely tests halogenated POPs and pesticides primarily in food samples.

## 5. REFERENCES

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# ANNEX 1. THE IMPORTANCE OF BREASTFEEDING

**Breastfeeding is an ideal way to feed infants; its benefits go far beyond sound nutrition, and children should not be deprived of it without clear and compelling reasons.**

**Nutrition:** Breast milk provides, in an easily digested form, all the nutrients an infant need for the first six months of life. Breast-milk nutrients that other feeds may not provide include:

- high-quality protein
- long-chain polyunsaturated fatty acids, considered essential for the infant's developing brain and eyes
- micronutrients, including iron, in a form in which they are efficiently absorbed
- other factors necessary for optimal growth and protection against infection.

**Immunity:** From the moment of birth, breast milk actively protects infants against infection. It contains numerous anti-infective factors, including immunoglobulins and white blood cells, as well as growth factors that stimulate the development of the infant's gut. Studies show consistently that, even with optimal hygiene, the rate of diarrheal disease of artificially fed infants is several times that of breastfed infants; they also have higher rates of respiratory, ear and other infections. A study in a situation of poor hygiene found that the risk of death from diarrhoea in artificially fed infants was 14 times that of fully breastfed infants. Even in developed countries non-breastfed children have higher rates of diarrhoea. Some chronic diseases in later life, such as adult-onset diabetes, are also increased by lack of breastfeeding.

**Up to six months of life, breast milk alone provides all the fluids and nutrients that a child needs.** Exclusive breastfeeding (i.e., no other food or drinks given, not even water) for the first six months offers maximum protection to infants against pneumonia, diarrhoea and other common infections of childhood.

**Up to two years of age or more, breast milk continues to provide high-quality nutrients and helps protect against infection.** From 6 to 12 months, breast milk usually provides 60%–80% of all energy, protein and other nutritional requirements – e.g., vitamins and other micronutrients, and from 12 to 23 months, breastfeeding can provide up to 35%–40% of these requirements.

**Psychosocial development:** Breastfeeding promotes the emotional relationship, or bonding, between mother and child.

## ANNEX 2. SUMMARY

# INFORMATION ON HUMAN MILK SURVEYS JOINTLY ORGANIZED BY THE WHO AND UNEP

Based on previous surveys, mothers should be reassured that breast milk is naturally the superior food for infants. This survey is intended to monitor the effectiveness of a new international agreement to reduce the levels of certain chemicals in our environment and which appear in human milk. In ratifying this agreement, countries have signalled their commitment to assuring that present and future generations will enjoy safe and wholesome nutrition and other benefits that only pure breast milk can offer.

Persistent organic pollutants (POPs) are a group of chemicals that have been intentionally or unintentionally introduced and widely distributed in the environment. Due to their stability and fat solubility, they can accumulate in many fat-containing foods as well as the human body, where traces of POPs can be found in human milk. Some of the most commonly detected POPs are organochlorine pesticides, such as DDT, industrial chemicals, most notably polychlorinated biphenyls (PCBs) and Short-chain and Medium-chain Chlorinated Paraffins (SCCPs and MCCPs), and industrial by-products, especially dioxins (PCDDs and PCDFs). These chemicals as a group have been of public health concern. For many years, the World Health Organization (WHO) has collaborated with countries in the development of data on levels of POPs in food as well as human milk. This data has been used to assess the risks to human health posed by exposure to various POPs. In 2001, an international agreement, the Stockholm Convention on POPs, was adopted by a large majority of the world's countries to reduce the amount of these substances in the environment and in people, and in 2004 the convention entered into force.

Meeting under the auspices of the UNEP, parties to the Convention have identified human milk as one of the core matrices to be monitored to evaluate the effectiveness of the Stockholm Convention in reducing emissions of POPs. In conducting the surveys of POPs in human milk as joint WHO/UNEP programmes, monitoring the POPs covered by the Stockholm Convention assist countries in their planning, management and evaluation of their POPs-reduction plans. These surveys will also promote human milk as the optimal food for infants as it will be the basis for possible source-directed measures to reduce levels of POPs in human milk. This is consistent with the Global Strategy for Infant and Young Child Feeding, endorsed by the World Health Assembly and the UNICEF Executive Board in 2002. The surveys include samples from various regions of the world and will reflect different food consumption patterns.

This survey will include at least 50 first-time mothers whose milk samples will be analysed for POPs. The average values for the various POPs will be used in reports. Individual results with the names of donors are considered confidential and will not be reported. This survey will be repeated periodically about every 4 to 5 years with another group of first-time mothers and the average values of the two groups will be compared to give an indication of the changes, if any, in the levels of POPs. It is anticipated that levels of POPs in human milk will show downward trends as countries implement measures to reduce the emission of POPs into the environment.

At the same time, evidence for the health advantages of breastfeeding has continued to increase. On a population basis, exclusive breastfeeding for six months is the recommended feeding mode for the vast majority of infants, followed by continued breastfeeding with appropriate complementary foods for up to two years or beyond.

# ANNEX 3. GUIDANCE FOR MOTHERS COLLECTING MILK SAMPLES AT HOME

**Goal of sampling:** The goal of this sampling exercise is to collect a sample of your milk in a way that avoids unnecessary contamination.

**How to collect samples:** You may collect the sample preferably by using manual expression. You have already been given instructions on these methods, but remember:

- You should not use any other vessel for collecting milk. You must not use cups or other bottles you may have at home. You should collect your milk directly into the small HDPE bottle provided to you.
- You should keep your breasts and hands as clean as possible, but soap should be avoided because they may contain chemicals that interfere with the analysis. When it is necessary to use soap, you should rinse your breasts and hands thoroughly with clean water to wash off any traces of soap.
- You should avoid using ointments on your nipples before collecting your milk. If you have used ointment on the sampling day, you must wash your nipples with soap and then thoroughly rinse with clean water.

The following tips are provided to make expression and collection of your milk easier, faster, and more comfortable:

**Manual method:** If you wish to manually express your milk, you should collect it directly into the provided collection container.

**When to collect your sample:** It is recommended that you collect your sample at the regular feeding time, usually two hours after the previous nursing. You should try to collect hind-milk, which is the milk expressed towards the end of each feeding.

**Storage and transport of your sample:** If you do not collect 50 ml at once, the partial sample may be stored in the refrigerator and sampling can be continued the next day. If 50 ml is still not collected, the sampling may be continued for a third day. However, after 3 days, sampling should be stopped and the collected sample should be frozen if possible. The sample should be delivered to the place that the National Coordinator has identified, as soon as possible and protected from high temperatures during the transport. However, you should best collect your sample in one day and return it to the designated clinic the next day.

# ANNEX 4. MODEL INFORMED CONSENT FORM

## Certificate of Consent

**I have been invited to take part in the research on UNEP Global Survey of Human Milk for Persistent Organic Pollutants (POPs). I have been told the purpose and procedures of this survey, in summary:**

## Purpose of the survey

Persistent organic pollutants (often called POPs) are a group of man-made chemicals which can be found in the environment. These chemicals are persistent over time, and they often are found in fat-containing foods, including human milk. The World Health Organization (WHO) GEMS/Food Programme is helping many countries around the world to conduct surveys to measure levels of POPs in human milk. WHO and UNEP have jointly performed surveys that will help to determine if levels of POPs are going down because of the Stockholm Convention. This survey will also support and strengthen national capabilities for the monitoring and sound management of POPs in food.

While concerns about POPs have been raised, the evidence for the health advantages of breastfeeding has continued to increase. On a population basis, exclusive breastfeeding for six months is the recommended feeding mode for the vast majority of infants, followed by continued breastfeeding with appropriate complementary foods for up to two years or beyond.

## Procedures

We are asking you to give one 50 ml sample of your milk. The milk can be collected using either manual expression or a breast pump. The sample will be collected at the most convenient health clinic or in your home. Your sample will be analysed for selected POPs and will also be mixed with samples from at least 25 other mothers for analysis.

These results may also be combined with those of other countries to give a regional assessment.

## Risks and discomforts

You may have some discomfort when you express your milk by hand. None of the questions that we will ask will be personal.

## Confidentiality

The information that we collect from this research project will be kept confidential. Informa-

tion about you that will be collected from the survey will be stored in a file that will not have your name on it, but a number assigned to it instead. The name associated with the number assigned to each file will be kept under lock and key and will not be divulged to anyone except ..... **[Insert name of National Coordinator]**.

Regarding inadvertent disclosure, the consequences are not expected to be significant because your results will not include your name and will be identified by a code. In addition, only average (mean) results will be reported and not those of any individual.

#### Alternatives to participation

You do not have to take part in this research if you do not wish to do so, and refusing to participate will not affect your treatment at this centre in any way. You will still have all the benefits that you would otherwise have at this centre.

You may stop participating in the research at any time that you wish until your sample has been pooled with other samples; if you choose to end your participation, you will not lose any of your rights as a patient here. Your treatment at this centre will not be affected in any way.

#### Contact information

If you have any questions, you may ask them now or later. If you wish to ask questions later, you may contact the following person: ..... **[Insert name and contact information for the National Coordinator]**

**I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked, have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study until my sample has been pooled with others. If I choose to withdraw from the study, I understand that I can do so without in any way affecting my medical care. I also consent that any excess sample of breast milk may be kept for related surveys in the future.**

Print Name of Participating Mother

Date and Signature of Participating Mother

\_\_\_\_\_

\_\_\_\_\_

\_\_\_/\_\_\_/\_\_\_ (dd/mm/yy)

#### **If illiterate**

#### **Print Name of Independent Literate Witness Date and Signature of Witness**

(If possible, this person should be selected by the participant and should have no connection to the research team)

\_\_\_\_\_

\_\_\_\_\_

\_\_\_/\_\_\_/\_\_\_ (dd/mm/yy)

Print Name of Researcher

Date and Signature of Researcher

\_\_\_\_\_

\_\_\_\_\_

\_\_\_/\_\_\_/\_\_\_ (dd/mm/yy)

## **ANNEX 5. SUMMARY INFORMATION FOR A POOLED SAMPLE**

## ANNEX 5. Summary information for a pooled sample

| Fifth UNEP/WHO Coordinated Survey of Human Milk for Persistent Organic Pollutants                                                                                                                                                      |                          |                                                                                                                 |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------|
| SUMMARY INFORMATION FOR A POOLED SAMPLE<br>(Based on confidential questionnaires from mothers donating human milk samples)                                                                                                             |                          |                                                                                                                 |
| Country                                                                                                                                                                                                                                | Pool Identification code | Number of mothers in the pool                                                                                   |
| <b>1. Ages of the mothers</b><br><br>Mean <input type="text"/><br><br>Range <input type="text"/>                                                                                                                                       |                          | <b>2. Mother's height (in cm)</b><br><br>Mean <input type="text"/><br><br>Range <input type="text"/>            |
| <b>3. Mother's weight before pregnancy (in kg)</b><br><br>Mean <input type="text"/><br><br>Range <input type="text"/>                                                                                                                  |                          | <b>4. Child's age at sampling (in weeks)</b><br><br>Mean <input type="text"/><br><br>Range <input type="text"/> |
| <b>5. Area of residence during last 10 years: (% of total mothers in the pool)</b><br><br>Urban <input type="text"/> Rural <input type="text"/>                                                                                        |                          |                                                                                                                 |
| <b>6. Mother's dietary habits (% of total mothers in the pool)</b><br><br>Mixed diet <input type="text"/> Vegetarian but with milk and egg <input type="text"/><br>Strictly vegetarian <input type="text"/> Other <input type="text"/> |                          |                                                                                                                 |

|                                                                                                                                                                                                             |                                                                                                                                                                                                                      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>7. Mother born in the country</b><br>(% of total mothers in the pool)<br><div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 5px;"></div>                                        | <b>8. Mothers who were breastfed in infancy</b><br>(% of total mothers in the pool)<br><div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 5px;"></div>                                      |
| <b>9. Mother's mother born in the country</b><br>(% of total mothers in the pool)<br><div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 5px;"></div>                               | <b>10. Mothers working before pregnancy</b><br>(% of total mothers in the pool)<br><div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 5px;"></div>                                          |
| <b>11. Exposure to DDT from indoor house spraying to prevent mosquitoes</b><br>(% of total mothers in the pool)<br><div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 5px;"></div> | <b>12. Mothers with current weight being less than their weight before pregnancy</b><br>(% of total mothers in the pool)<br><div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 5px;"></div> |

| 13. Mother's consumption of food (% of mothers in the pool) |                                          |                                        |                                                                    |                                                             |                                                      |      |
|-------------------------------------------------------------|------------------------------------------|----------------------------------------|--------------------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------|------|
|                                                             | Fish and fish products (e.g. tuna salad) | Marine mammals (e.g. whales, dolphins) | Seafood other than fish and marine mammals (e.g. shrimps, mussels) | Milk and milk products (e.g. cheese, butter, cream, yogurt) | Meat and poultry and derived products (e.g. sausage) | Eggs |
| Never                                                       |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Less than once a week                                       |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Once a week                                                 |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Twice or less a week                                        |                                          |                                        |                                                                    |                                                             |                                                      |      |
| More than twice a week but not every day                    |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Every day                                                   |                                          |                                        |                                                                    |                                                             |                                                      |      |

| 14. Type of fish most often consumed by the mother (% of mothers in the pool)                                        |                                                                                                               |                                                                                                         |
|----------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| <b>Fish from the sea</b><br><div style="border: 1px solid black; height: 20px; width: 80%; margin: 5px auto;"></div> | <b>Fresh fish</b><br><div style="border: 1px solid black; height: 20px; width: 80%; margin: 5px auto;"></div> | <b>Both</b><br><div style="border: 1px solid black; height: 20px; width: 80%; margin: 5px auto;"></div> |

|                   |                                  |           |
|-------------------|----------------------------------|-----------|
| Date (dd/mm/yyyy) | Name of the National Coordinator | Signature |
|-------------------|----------------------------------|-----------|

## **ANNEX 6. QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS**

## **ANNEX 6. Questionnaire for potential human milk donors**

|                                                                                                            |              |                              |
|------------------------------------------------------------------------------------------------------------|--------------|------------------------------|
| UNEP-coordinated Survey of Human Milk for Persistent Organic Pollutants<br><br><b><i>CONFIDENTIAL!</i></b> |              |                              |
| <b>Questionnaire for the Potential Human Milk Donors</b>                                                   |              |                              |
| <b>Section 1: Personal Information</b>                                                                     |              |                              |
| Name                                                                                                       | Phone number | Today's Date<br>(dd/mm/yyyy) |
|                                                                                                            | e-mail       |                              |
| Address                                                                                                    |              |                              |

|                                                                                                                                                                                                                                                                     |                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| <b>Section for the National Coordinator</b>                                                                                                                                                                                                                         |                          |
| Individual Identification Code                                                                                                                                                                                                                                      | Pool Identification Code |
| Based on the established criteria, is the donor eligible?<br><br>Yes <input type="checkbox"/> No <input type="checkbox"/>                                                                                                                                           |                          |
| What is the donor's status with regard to the survey?<br><br>Selected <input type="checkbox"/> Reserve <input type="checkbox"/> Not Selected <input type="checkbox"/>                                                                                               |                          |
| If this mother has been pre-selected to donate a sample (or is designated as an alternate), the top of Section 4 should be completed and detached from this questionnaire. Section 4 should be sent to the clinic to be completed at the time of sample collection. |                          |

| Section 2: Donor Screening Questionnaire                                                                        |                                    |
|-----------------------------------------------------------------------------------------------------------------|------------------------------------|
| Name of the Interviewer:                                                                                        | Date of interview<br>(dd/mm/yyyy): |
| Place of interview:                                                                                             |                                    |
| 1. Are you planning to breastfeed your child?                                                                   |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/>        |
| 2. Is this your first child?                                                                                    |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/>        |
| 3. Are you expecting a single child? (not twins)                                                                |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/>        |
| 4. Are you having a normal healthy pregnancy?                                                                   |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/>        |
| 5. Have you lived at your current place of residence (city and country) for 10 years?                           |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/> *      |
| If no, number of years _____                                                                                    |                                    |
| 6. Are you under 30 years of age?                                                                               |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/> *      |
| If no, date of birth _____ (dd/mm/yyyy)                                                                         |                                    |
| 7. Do you live near incinerators, pulp and paper industries, metal industries, or where chemicals are produced? |                                    |
| Yes <input type="checkbox"/>                                                                                    | No <input type="checkbox"/>        |

**\*Note that if the answers to questions 5 or 6 was "No", please ask for the donor's actual residence time and/or birth date.**

**Instruction to the interviewer:** If any answers to questions 1–6 is "No" or if the answer to question 7 is "Yes", the donor is not eligible to participate in this survey. Please thank the donor for her interest and end the interview at this point.

**If all answers to questions 1–6 are 'Yes' and the answer to question 7 is 'No', proceed to Section 3.**

| <b>Section 3: Health History Questionnaire</b>                                                                                                                                                                                                                                                                                                           |                                          |                                        |                                                                    |                                                             |                                                      |      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|----------------------------------------|--------------------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------|------|
| Date of Birth (dd/mm/yyyy)                                                                                                                                                                                                                                                                                                                               |                                          |                                        |                                                                    |                                                             | Age                                                  |      |
| Height (cm)                                                                                                                                                                                                                                                                                                                                              |                                          |                                        | Weight before pregnancy (kg)                                       |                                                             |                                                      |      |
| 1. What is your expected delivery date (dd/mm/yyyy)?                                                                                                                                                                                                                                                                                                     |                                          |                                        |                                                                    |                                                             |                                                      |      |
| 2. Where have you been residing during the last 10 years:<br><br><div style="text-align: center;">             Urban (city) <input type="checkbox"/> <span style="margin-left: 200px;">Rural (countryside) <input type="checkbox"/></span> </div>                                                                                                        |                                          |                                        |                                                                    |                                                             |                                                      |      |
| 3. How would you describe your dietary habits before pregnancy?<br><br>Mixed diet <input type="checkbox"/> Vegetarian but with milk and eggs <input type="checkbox"/> Strictly vegetarian <input type="checkbox"/> Other <input type="checkbox"/>                                                                                                        |                                          |                                        |                                                                    |                                                             |                                                      |      |
| 4. How often, on average, did you eat following foods before pregnancy?                                                                                                                                                                                                                                                                                  |                                          |                                        |                                                                    |                                                             |                                                      |      |
|                                                                                                                                                                                                                                                                                                                                                          | Fish and fish products (e.g. tuna salad) | Marine mammals (e.g. whales, dolphins) | Seafood other than fish and marine mammals (e.g. shrimps, mussels) | Milk and milk products (e.g. cheese, butter, cream, yogurt) | Meat and poultry and derived products (e.g. sausage) | Eggs |
| Never                                                                                                                                                                                                                                                                                                                                                    |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Less than once a week                                                                                                                                                                                                                                                                                                                                    |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Once a week                                                                                                                                                                                                                                                                                                                                              |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Twice a week                                                                                                                                                                                                                                                                                                                                             |                                          |                                        |                                                                    |                                                             |                                                      |      |
| More than twice a week but not every day                                                                                                                                                                                                                                                                                                                 |                                          |                                        |                                                                    |                                                             |                                                      |      |
| Every day                                                                                                                                                                                                                                                                                                                                                |                                          |                                        |                                                                    |                                                             |                                                      |      |
| 4.1 What types of fish do you consume most often?<br><br><div style="text-align: center;">             Fish from the sea <input type="checkbox"/> <span style="margin-left: 100px;">Freshwater fish <input type="checkbox"/></span> <span style="margin-left: 100px;">Both <input type="checkbox"/></span> </div><br>Please state the species if known : |                                          |                                        |                                                                    |                                                             |                                                      |      |

|                                                                                                                                                                                                                                                                                                   |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p>5. Was your mother born in this country?</p> <p style="text-align: right;">Yes <input type="checkbox"/>                      No <input type="checkbox"/></p>                                                                                                                                   |
| <p>6. Were you breastfed?</p> <p style="text-align: right;">Yes <input type="checkbox"/>              No <input type="checkbox"/>      Do not know <input type="checkbox"/></p> <p>If you know, for how long? _____</p>                                                                           |
| <p>7. Were you engaged in work other than housework before pregnancy?</p> <p style="text-align: right;">Yes <input type="checkbox"/>                      No <input type="checkbox"/></p> <p style="margin-top: 20px;">If yes, please state the duration and describe type of work:</p>           |
| <p>8. Has the inside of your house been sprayed with DDT in order to prevent mosquitoes?</p> <p style="text-align: right;">Yes <input type="checkbox"/>                      No <input type="checkbox"/>                      Do not know <input type="checkbox"/></p> <p>If yes, when? _____</p> |

**Instructions to interviewer:**

**If this is a prenatal interview, the questionnaire with Sections 1-3 completed should be sent to the National Coordinator at this point for review.**

**If this is a postnatal interview and the breast milk will be collected today, proceed to fill Section 4.**

| To be completed by the National Coordinator if using pre-selection option                                                                                         |                       |                               |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------------------------|
| Mother's Name                                                                                                                                                     | Phone number<br>Email | Date of delivery (dd/mm/yyyy) |
| Address                                                                                                                                                           |                       |                               |
| Status of donor in regard to the survey<br><div style="text-align: right;">Selected <input type="checkbox"/>              Reserved <input type="checkbox"/></div> |                       |                               |
| Individual Identification Code                                                                                                                                    |                       |                               |

| <b>Section 4. To be completed by the Sample Collector</b>                                                                                                                                                                                                                                                                          |                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Name of the sample Collector:                                                                                                                                                                                                                                                                                                      | Date of sampling (dd/mm/yyyy): |
| Clinic of Collection:                                                                                                                                                                                                                                                                                                              | Place of collection:           |
| <b>Postnatal Information (to be taken at the time of sampling)</b>                                                                                                                                                                                                                                                                 |                                |
| 1. Are you prepared and agree to sign the consent form?<br><div style="text-align: center;">Yes <input type="checkbox"/>      No <input type="checkbox"/></div> If yes, attach signed consent form. If no, the donor is not eligible to participate in the survey.                                                                 |                                |
| 2. How old is your infant?<br><br>less than 3 weeks* <input type="checkbox"/> 3-4 weeks <input type="checkbox"/> 5-8 weeks <input type="checkbox"/> more than 8 weeks** <input type="checkbox"/>                                                                                                                                   |                                |
| 3. What is the sex of your infant?<br><br><div style="text-align: center;">Male <input type="checkbox"/>      Female <input type="checkbox"/></div>                                                                                                                                                                                |                                |
| 4. Is your current weight different than your weight before pregnancy?<br><br><div style="text-align: center;">Gained <input type="checkbox"/>      Lost <input type="checkbox"/>      Not changed <input type="checkbox"/></div>                                                                                                  |                                |
| 5. Can you provide a sample now?<br><br><div style="text-align: center;">Yes <input type="checkbox"/>      Later <input type="checkbox"/> When? _____      At home <input type="checkbox"/></div> If you want to take the sample at home, do you have a refrigerator? Yes <input type="checkbox"/> No <input type="checkbox"/> *** |                                |

**\* Infant must be more than 3 weeks (21 days) old. The collector should advise the mother to return after the infant is 3 weeks old for milk sampling.**

**\*\* Sample must be collected within 3 to 8 weeks after delivery. Do not take the sample. Inform National Coordinator of the situation.**

**\*\*\* The sample may be stored in the refrigerator at about 4 °C for a maximum of 72 hours, or for longer times in the freezer at -20°C.**

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