Global Monitoring Plan on Persistent Organic Pollutants

Guidelines for Organization, Sampling and Analysis of Human Milk on Persistent Organic Pollutants

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UNEP-coordinated Survey of Human Milk for Persistent Organic Pollutants

Guidelines for Organization, Sampling and Analysis

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Acronmys and Abbreviations

°C	Degree Celsius
%	per cent
COP	Conference of the Parties
DDE	Dichlorodiphenyldichloroethylene, metabolite of DDT
DDT	Dichlorodiphenyltrichloroethane
DTIE	Division of Technology, Industry and Economics
EC	European Commission
ECD	Electron capture detector
EU	European Union
GEF	Global Environment Facility
GMP	Global Monitoring Plan
HBCD	Hexabromocyclododecane
HCB	Hexachlorocyclobenzene
HRGC	High-resolution gas chromatography
HCH	Hexachlorocyclohexane
HRMS	High-resolution mass spectrometry
(P)BDE	(Poly)brominated diphenyl ethers
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxins
PCDF	Polychlorinated dibenzofurans
PFOS	Perfluorooctanesulfonic acid
POP(s)	Persistent organic pollutant(s)
QA	Quality assurance
QC	Quality control
SC	Stockholm Convention
UNEP	United Nations Environment Programme
UNICEF	United Nations Children's Fund
WHO	World Health Organization

1 INTRODUCTION

The Stockholm Convention on Persistent Organic Pollutants (POPs)[1] entered into force on 17 May 2004. The objective of the Convention is the protection of human health and the environment from POPs by reducing or eliminating releases into the environment. Parties have agreed that they need a mechanism to measure whether this objective is achieved. According to Article 16 of the Convention, its effectiveness shall be evaluated starting four years after the date of entry into force of the Convention and periodically thereafter at intervals to be decided by the Convention of Parties (COP). In order to achieve this goal, a guidance document on the Global Monitoring Plan (GMP) was prepared by UNEP [2], which has been updated and amended to include new POPs at later conferences of the parties.

Participants in a series of meetings convened by UNEP Chemicals on this issue have consistently recommended that human milk should be one of the media to be monitored. Human milk contains many lipid soluble compounds that are also present in mother's adipose tissue. Therefore, it can also be assumed that the concentrations of lipophilic contaminants in human milk are representative for those in plasma, serum lipid and adipose tissue. Thus, concentrations of these contaminants in human milk do reflect the body burden and can thus be used as an indicator for the overall exposure of the general population. The collection of human milk is a non-invasive sampling method, offering several advantages over the collection of other biological samples to assess overall human exposure. In addition, the high content of fat makes the extraction method easier and the precision of the measurements higher.

The World Health Organization (WHO) has performed exposure studies on concentrations of specific POPs in human milk at the global level since the end of the 1980es. The main objectives of these surveys were: 1) to produce more reliable and comparable data on concentrations of certain POPs in human milk for further improvement of health risk assessment in infants, 2) to provide an overview of exposure levels in various countries and geographical areas, 3) to identify highly exposed local populations in relation to their daily intake for guidance on risk management actions, including epidemiological follow-up studies; and 4) to promote, if necessary, additional national studies to be closely linked with the respective studies through the use of the same protocol.

Table 1. Overview of participating countries in different human milk surveys

1st Round	1987-1988	WHO	12 countries
2nd Round	1992-1993	WHO	19 countries
3rd Round	2000-2003	WHO	26 countries
4th Round	2004-2007	WHO/UNEP	13 countries
5th Round	2008-2011	WHO/UNEP	45 countries
6th Round	2012-2015	UNEP	17 countries

[1] UNEP (2001): United Nations Environment Programme, Stockholm Convention on Persistent Organic Pollutants (POPs), Text and Annexes, http://chm.pops.int

[2] UNEP (2015): Guidance on the Global Monitoring Plan for Persistent Organic Pollutants. **UNEP**/POPS/COP.7/INF/39, February 2015.

2 AIM OF THESE GUIDELINES

The objective of these guidelines is to give a framework for all activities and tasks associated with organization, sampling and analysis of human milk samples. Experiences from the previous WHO/UNEP coordinated exposure studies were used and improved where necessary. Therefore, the 2007 guidelines of WHO for developing a National Protocol [3] supporting the global human milk studies were amended taken into account practical considerations but also the additional listing of new POPs. This guideline represents an update of the previous guidelines "UNEP-coordinated Survey of Mothers' Milk for Persistent Organic Pollutants - Guidelines for Organization, Sampling and Analysis" (UNEP Chemicals 2012a) and its Annexes (UNEP Chemicals 2012b), and "Standard Operating Procedure for POP Sampling in Breast Milk" (UNEP, BCCC Uruguay and Fiocruz, 2010).

3 HEALTH BENEFITS OF BREASTFEEDING

No efforts by governments, the World Health Organization, UNICEF and other international organizations should be undermined to promote exclusive breastfeeding below six months of age with continued breastfeeding up to two years. All persons involved in these surveys, but especially those who have direct contact with potential donors, should be well informed about the health benefits of breastfeeding for the infant as well as the mother (see Annex 1).

While national protocols may require flexibility, the following general principles should be observed:

- Breastfeeding should be protected, promoted and supported.
- The health benefits of breastfeeding to both mother and baby 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.
- Ethical review, including prior informed consent, should be respected.
- Safeguarding of confidential information should be assured.
- Quality assurance of results should be independently confirmed.

[3] Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP, Guidelines for Developing a National Protocol (Revised 1 Oct 2007)

4 IMPLEMENTATION ASPECTS OF HUMAN MILK SURVEYS

4.1 Overview on Participating Countries in UNEP/GEF-GMP-2 projects

The following Table 2 lists the projects and participating countries:

Table 2: Projects and countries participating in the UNEP mothers milk study 2016 - 2019 (GEF-GMP2)

Region	Country	Country(abbr)
Pacific Islands	Fiji	FJI
	Kiribati	KIR
	Marshall Islands	MHL
	Niue	NIU
	Palau	PLW
	Samoa	WSM
	Solomon Islands	SLB
	Tuvalu	TUV
	Vanuatu	VUT
Africa	DR Congo	COD
	Egypt	EGY
	Ethiopia	ETH
	Ghana	GHA
	Kenya	KEN
	Mali	MLI
	Mauritius	MUS
	Morocco	MAR
	Nigeria	NGA
	Senegal	SEN
	Tanzania	TZA
	Togo	TGO
	Tunisia	TUN
	Uganda	UGA
	Zambia	ZMB
GRULAC	Antigua and Barbuda	ATG
	Argentina	ARG
	Barbados	BRB
	Brazil	BRA
	Chile	CHL
	Colombia	COL
	Ecuador	ECU

	Jamaica	JAM
	Mexico	MEX
	Peru	PER
	Uruguay	URY
Asia-Pacific	Cambodia	KHM
	Indonesia	IDN
	Lao PDR	LAO
	Mongolia	MNG
	Philippines	PHL
	Thailand	THA
	Vietnam	VNM

For each country, a National Coordinator is identified being responsible for the overall planning and implementation of the survey in the country 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 subjects.

The continuous exchange of information between UNEP, the expert Laboratory and the national coordinators on basic information such as nomination and contact addresses is a prerequisite for a successful organization of a study on a global level. UNEP and the expert Laboratory should have a permanently updated list of participants and contact points available and continuously exchange relevant information. The national coordinators should establish close contacts to UNEP and the expert laboratories for exchange of information on the status of collection of samples, in particular with regard to the coordination of the shipment.

4.2 Concept of the Survey

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

4.2.1 General Goals

The samples should be representative for a country. For this, criteria for selection of donors of human milk were developed.

For Article 11 of Stockholm Convention it is important to identify priority POPs in the population. For the purpose of Article 16 of Stockholm Convention, the need to quantify concentrations of POPs over time makes it essential that comparable cohorts can be identified so that in the following years statistically reliable evaluation of time trends can be performed. In certain countries it might be of interest to collect individual samples in different regions. In addition, variations in dietary patterns or locally contaminated areas might 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 these features in their protocols, provided the main aims of the survey are not compromised.

For evaluation of time trends, participation in different sampling periods is necessary. The first sampling period is conducted to determine baseline concentrations for POPs in randomly

selected individual samples of human milk and pooled samples made from them. A second sampling period should be conducted with a similarly selected cohort e.g. four or five years later (or other time period deemed appropriate). Future samplings should be undertaken at regular intervals and the monitoring of human milk for POPs considered a long-term activity.

4.2.2 General Idea of Collection of Individual Samples, Preparation of Pooled Samples and Role of Expert Laboratories

In order to get statistically reliable data, an appropriate number of individual donors must be recruited to provide samples for the survey. Chapter 4.3 provides detailed information on the appropriate number of these individual samples and selection of donors.

Equal aliquots of these individual samples are mixed to form a composite sample ("pooled sample"). In the WHO-coordinated exposure studies of the 1980ies and 1990ies on PCDD, PCDF and dioxin-like PCBs, only pooled samples were used in the monitoring of human milk because most laboratories, even in developed countries, could not adequately analyse these POPs. The analysis of pooled human milk samples is also far less expensive than the analysis of individual samples. In addition, it is easier for each donor to provide the lower volume of milk required for pooled analyses.

To ensure the reliability of exposure data and to improve comparability of analytical results from different laboratories, WHO has coordinated a number of inter-laboratory quality assessment studies. The fourth round on levels of PCB, PCDD and PCDF in human milk was conducted between February 1996 and April 1997. The objective was to identify laboratories, whose results could be accepted by WHO for exposure assessment studies. The final report presents the results of the study and a list of accepted laboratories for each of the studied compounds. As only the State Institute for Chemical and Veterinary Analysis of Food (Freiburg, Germany) met all criteria for analyses of PCDD, PCDF, dioxin-like PCB, indicator PCB and fat in human milk, this laboratory was selected as reference laboratory for the following WHO exposure studies. This laboratory was later qualified as the European Union Reference Laboratory (EU-RL) for Dioxins and PCB in Feed and Food and as the EU-RL for Pesticide Residues in Food of Animal Origin and Commodities with High Fat Content and was selected as expert laboratory for the exposure studies jointly organised by WHO and UNEP since 2005.

In addition to CVUA Freiburg as expert laboratory for lipophilic POPs, one expert laboratory was chosen for PFOS (Örebro University). By performance of analysis of the pooled samples (being representative for the participating countries) in the rexpert laboratory, a high degree of reliability of the analytical results can be achieved. Such data are essential to statistically validate changes in concentrations of POPs over time. Therefore, the use of representative pooled samples with **determination of all analytes of interest** (see chapter 4.2.3) **in the expert laboratory** has continuously been a key element to monitor concentrations of the relevant POPs of the Stockholm Convention in human milk.

The analysis of the **individual samples** (from the individual donors) can provide information on the distribution of exposures and on factors possibly contributing to exposure. Since 2007 the WHO/UNEP guidelines recommend the analysis of individual samples for the **basic pesticide POPs and indicator PCB in the participating country**. These compounds can be determined by analytical methods requiring basic instrumentation available in many developing countries, such as HRGC with ECD.

For quality control purposes, the mean results of individual analyses can be compared with the result of the pooled sample analysed by the expert Laboratories because the average value from individual samples should be equal to the pooled sample value.

See also chapter 5 on analysis of the samples.

4.2.3 Analytes of interest for determination in representative pooled national human milk samples respectively individual samples

Table 3 presents the POPs listed in the Stockholm Convention with status of 2013 (parent compounds and relevant transformation products). These have to be determined in the representative pooled national human milk samples by the expert laboratory.

Table 3: POPs listed in the Stockholm Convention (parent compounds and relevant transformation products) to be determined by the expert laboratory in the representative pooled national human milk samples

Compounds to be analysed in pooled national mothers milk samples by CVUA	
Initial POPs	
Aldrin	Aldrin
Chlordane	*cis*- and *trans*-chlordane; and *cis*- and *trans*-nonachlor, oxychlordane
DDT	4,4'-DDT, 2,4'-DDT and 4,4'-DDE, 2,4'-DDE, 4,4'-DDD, 2,4'-DDD
Dieldrin	Dieldrin
Endrin	Endrin
HCB	HCB
Heptachlor	Heptachlor and heptachlorepoxide
Mirex	Mirex
PCB	ΣPCB_6 (6 congeners): 28, 52, 101, 138, 153, and 180
	PCB with TEFs* (12 congeners): 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189
PCDD/PCDF	2,3,7,8-substituted PCDD/PCDF (17 congeners)
Toxaphene	Congeners P26, P50, P62

* PCB with TEFs (Toxic Equivalency Factors) assigned by WHO in 1998

POPs listed at COP-4	
Chlordecone	Chlordecone
α-HCH	α-HCH
β-HCH	β-HCH
γ-HCH	γ-HCH
Hexabromobiphenyl	PBB 153
Pentachlorobenzene	PeCBz
c-penta BDE	BDE 47, 99, 153, 154, 175/183 (co-eluting)
c-octa BDE	Optional: BDE 100
POPs listed at COP-5	
Endosulfan	α-, β-endosulfan; and endosulfan sulfate
POPs listed at COP-6	
HBCD	α-HBCD, β-HBCD, γ-HBCD

The individual samples can/should be analysed for analytically basic POPs ("initial POPs" except PCDD/PCDF and, if possible, HCH) at a laboratory selected by the National Coordinator. Ideally this laboratory should be located in the country. Emphasis should be on analytical proficiency as demonstrated by adequate quality assurance procedures and

confirmed by successful participation in inter-laboratory studies for pesticide POPs and indicator PCB. If such a laboratory is not available in a participating country, capacity building might be supported by other programmes.

4.3 Developing a National Protocol

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

In order to get 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 primiparae mothers should be available from the health statistics office. However, it is recognized that some flexibility may be necessary for countries with small populations and/or low birth rates. If this is a problem, extending the sample collection period should be considered as the first option to increase the number of available 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 the inclusion of more than 50 individual samples and is encouraged. In particular, countries with populations greater than 50 million should include at least one additional participant per one million population over 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 within the responsibilities of the National Coordinator to make sure that the number of samples being collected for analysis can provide a sufficient statistical base to allow scientifically valid assessments of changes in concentrations of POPs over time.

4.3.1 Selection of Donors

The national survey protocol should be developed to assess the concentrations and changes over time of POPs for a defined cohort of the country. For many countries, collection of samples will be conducted at health clinics providing postnatal services. Therefore, selection of clinics may be as important as the selection of donors, particularly in regard to staff and facilities. As exposure to POPs is mainly through food, food consumption patterns and concentrations of POPs in those foods will mainly determine the concentrations of POPs in human milk. The location of residence, usually urban or rural, may also be associated with different exposure levels for certain POPs. Living in highly polluted areas, such as in the vicinity of incinerators, pulp and paper industries and metal industries or where organochlorine substances are produced or used, are also known to influence exposure to POPs. Persons with markedly different exposure to POPs should not be included in the survey to avoid skewing the results.

The most important selection criterion to be met by all donor mothers is:

- Mother should be *primiparae*.

Other criteria are recommended as follows:

- Mother should be under 30 years of age
  (The National Coordinator might consult national health statistics for possible advice on

setting the maximum age to assure a sufficient number of potential donors. In order to further reduce variability, an age range might 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.*, no twins).
- Mother should have resided in the represented area (country) for at least the previous ten years.
- Mother should not reside in local areas where 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, there are two general procedures to identify possible donors. They are:

Selection before giving birth: 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 be encouraged to breastfeed even if they do not intend to or are not selected to participate in the survey. Once a participant indicates a willingness to take part in the survey, she should be invited to complete Sections 1-3 of the questionnaire (see Annex 2). In addition, the informed consent form might also be completed at this time. The questionnaire can be completed through a personal interview at the prenatal clinic or completed by the potential donor at home and returned to the clinic, either in person or by mail. National Coordinators should decide on the best 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 participants. Participants should be notified of their selection and where and when the sample will be collected. For most countries, 50 potential donors should be selected. In addition, 10 reserve donors should be identified in case some selected donors are unavailable. Criteria for selecting participants are discussed in Section 4. Note that those not selected for the survey should be informed and thanked for their time and interest.

Selection after giving birth: It is also possible to collect samples after the mother has given birth, i.e., without pre-selection as above. This is done at postnatal clinics and other venues, e.g., well-baby clinics. Mothers should be interviewed and Section 2 of the questionnaire should be completed (see Annex 2). If qualified, Sections 3-4 of the questionnaire should be completed and the mother should sign the informed consent form. Samples can then be collected, either immediately or later at home. While this method can reduce the time of the survey by up to 4 months, it does not allow for further stratification of the cohort to reduce variability. However, after the cohort selection criteria have been established from the first sample collection, this method offer advantages for the second and subsequent sample collections.

Each procedure has benefits and disadvantages, but the second is simplest and most efficient. Furthermore, this avoids a considerable delay of a project caused otherwise by the time and efforts to have 250 questionnaires completed from mothers contacted before giving birth for selection of 50 potential donors after giving birth. Therefore, with regard to the tight time frame, the selection of donors after giving birth is recommendable for these projects.

4.3.2 Interviewing Potential Donors

The model questionnaire for donors (see Annex 2) should be used as the basis for recording information from women about their participation in the study. Note that the National Coordinator should carefully review the questionnaire for applicability to the country. Special consideration should be given to the exclusion criteria contained in chapter 4.3.1. If necessary, these criteria should be modified to balance the need for a suitable pool of possible donors with the wish to reduce factors [4] that contribute to variability.

4.3.2.1 Interviews before Giving Birth

For selection before giving birth, initial interviews should be conducted at prenatal clinics at least 2 months prior to delivery. Interviewers should be aware of prenatal information on breastfeeding [5] as well as available local support through health services or in the community. The interviewer should first ascertain whether the woman plans to breastfeed her infant. For a woman who has not decided, this may be an opportunity to provide her with information about the benefits of breastfeeding for her and her 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 woman 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 'hot spots' and mothers 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[6]. The completed questionnaires should be sent to the National Coordinator for final selection. As they are confidential, the questionnaires and the information contained therein should be handled with care and according to applicable national requirements. Based on responses to Sections 1-3 of the questionnaire, the National Coordinator should develop selection criteria that would promote the most comparable and reliable survey results. In order to be able to determine changes in exposure levels over time, it is very important that the criteria for donating mothers be sufficiently robust to be repeatable during future surveys.

National Coordinators should review the questionnaires of the potential donors and select 50 potential donors that best meet the criteria for inclusion in the survey. In this regard, additional reserve donors, *e.g.,* 10 persons, should also be identified in the event that some selected donors are not available or otherwise drop out of the survey. Selected pregnant women should be notified of their inclusion in the survey and invited to provide a sample of their milk 3 to 8 weeks after the birth at a designated postnatal clinic.

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4 Harris CA, Woolridge MW and Hay AW (2001) Factors affecting the transfer of organochlorine pesticide residues to breastmilk. Chemosphere **43**, 243-56

5 Prenatal information as defined under step 3 of the WHO/UNICEF BFHI programme and in the related training course session 3 (pages 52–70)

6 A list of National POPs Contact Points and National Implementation Plans are available at the Stockholm Convention Secretariat Website at http://www.pops.int

4.3.2.2 Interviews after Giving Birth

Alternatively, donors may be selected after giving birth provided that they meet the basic criteria in Section 2 of the questionnaire. Once criteria have been fixed, this approach is perhaps the most simple, cost-effective and time-saving method for the identification of donors.

4.3.2.3 Questionnaire Collecting Relevant Information from Donors and Control of Fulfilment of the Criteria

If the woman fulfils the criteria and indicates she intends to primarily or exclusively breastfeed, the interviewer should generally explain the background and purpose of the survey as described in the summary information (see Annex 3). A copy of this information should be provided in the local language as well. If the woman indicates an interest in participating, Sections 1-2 of the questionnaire (see Annex 2) 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 woman is disqualified because of 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 the 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 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.

4.3.2.4 Handling of Questionnaires and Submission of Summary Information

The National Coordinator should retain questionnaires of all respondents until the end of the study. However, questionnaires of donors should be retained for future reference. Retention of all records should conform to national requirements and international norms concerning confidentiality. The National Coordinator should complete a summary of information form about mothers donating samples to the pooled sample to be submitted to UNEP (see Annex 7).

4.3.3 Suitable Glassware for Collection of Samples and Preparation of the Pooled Sample

The recommended glassware glassware for collection of samples is uncoloured laboratory glass bottles (*e.g.*, DIN thread 45) with crew caps made of PBT, complete with PTFE-protected seal (*e.g.*, DIN thread 45). The following Figure 1 shows an example.



Figure 1: Glassware for human milk sampling (typically 60 bottles of 100 ml, and 1 bottle of 2000 ml per country)[7]

Before shipment the glassware is washed in a dishing machine, rinsed with distilled water and heated overnight at 400 °C. After this procedure the glassware is ready for use.

Within the framework of the Project Cooperation Agreement (PCA) for the Project UNEP/GEF GMP2" between UNEP and CVUA Freiburg (2016) the CVUA Freiburg was asked by UNEP to supply glassware for the sampling of individual breast milk samples and receive the shipment of the pooled breast milk sample back from the participating country for POPs analysis. Thus, the CVUA Freiburg will be the primary contact for implementation of the human milk survey. The CVUA Freiburg will also handle the preparation of a sample aliquot to be shipped to Örebro University and subsequent PFOS analysis.

[7] As example, these bottles are available online from Duran glass: http://www.duran-group.com/en/products-solutions/laboratory-glassware



Figure 2: Example of packages of glassware for countries participating in the 6th round (2012-2015)

For customs, CVUA Freiburg had to attach a pro-forma invoice (see Annex 5) with a declaration of an appropriate value of the shipment in order to avoid payment of custom fees.

4.3.4 Collection of Samples

Sampling can be carried out between three to eight weeks (from 21 days to two months) after delivery. At the time of sample collection, individual interviews should be used to complete the information in the participant questionnaire (see Annex 2). Donors should already have also received verbal and written information concerning the survey. The procedures of the survey should be explained, particularly the rights of the donor to withdraw from the survey without prejudice. Following this, the donors should be requested to give their written consent on a standard *Informed Consent Form* (Annex 4). The sample can then be collected.

Mothers should provide the sample at the local contact place where collection can be

supervised. At least 50 ml of milk in total should be collected by hand expression [8] after a feeding or while infant is nursing on the other breast, to take advantage of the let-down reflex of the mother. If it is her wish, the mother may collect the sample at home, in which case manual expression is preferred. If so, she should be given detailed instructions for taking, storing and transporting of milk samples (see Annex 6). The person who gives the instructions should check the mother's understanding on how to proceed. Mothers should also be given a clean glass jar with a protected screw cap to collect and store the milk sample. Sample collection jars should be labelled with the donor's individual identification code and not the name of the mother.

4.3.5 Preservation of Collected Samples

4.3.5.1 By Freezing

The sample should be collected directly to the collecting jar and, if collected at home, stored in the collecting jar in the home freezer until it can be delivered. Otherwise milk samples 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.

4.3.5.2 By Addition of Potassium Dichromate

If refrigeration is not possible, individual and/or the pooled milk samples might be stabilized by potassium bichromate, particularly in countries with a hot climate and difficulties in an appropriate temperature control [9], [10].

The easiest way would be the addition of a small tablet of potassium dichromate ($K_2Cr_2O_7$) to chemically sterilize the milk. As example, for this purpose dichromate tablets are available in Germany (*e.g.,* Merck, Laborkatalog Merck, 2000, art.104858: "Kaliumdichromat-Tabletten zur Konservierung von Milchuntersuchungsproben" (potassium dichromate tablets for preservation of milk samples for analysis)), see the following picture. Characteristics of tablets: weight of 97 mg to 107 mg per tablet containing 32-24 mg dichromate per tablet.

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[8] Different WHO/UNICEF materials available at http://www.who.int/nutrition/publications/infantfeeding/en/

[9] Needham LL and Wang RY (2002) Analytical considerations for measuring environmental contaminants in breast milk - Chemical contaminants in breast milk: mini-monograph, Environmental Health Perspectives **110**, 317-324

[10] Schecter A, Pavuk M, Päpke O and Malisch R (2003) Potassium dichromate and ethyl alcohol as blood preservatives for analysis of chlorinated organics, Organohalogen Compounds 60:114-117

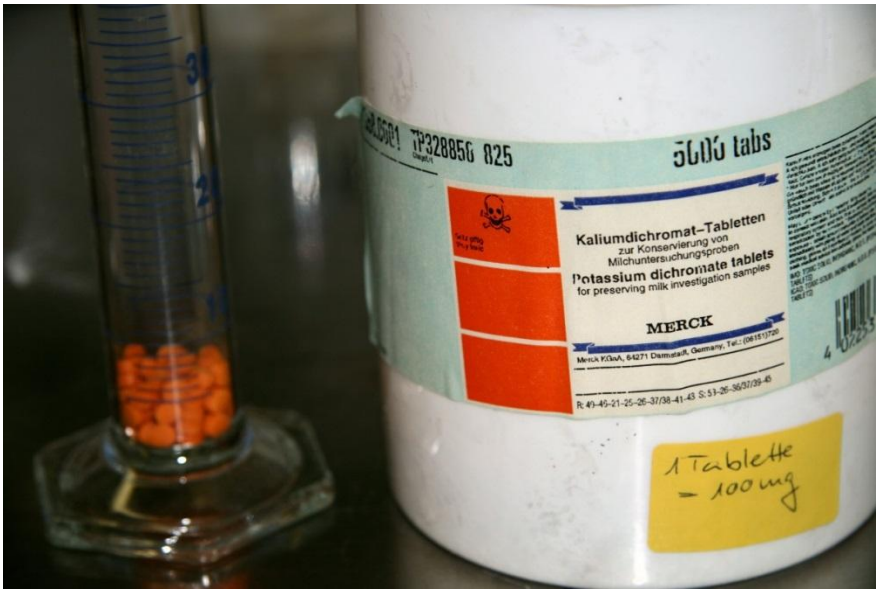


Figure 3: Example of potassium dichromate tablets for preservation of (human) milk samples

The addition of dichromate to milk should be 0.1 % which is equivalent to three tablets (= 100 mg dichromate) per 100 ml sample. This addition is a very comfortable way to add dichromate in a way which avoids irritant reactions otherwise easily caused by handling of pure potassium dichromate powder. However, under laboratory conditions, a classical way is possible, as well, for preparation of the pooled sample: With the usual precautions in a laboratory, one can add about 140 mg potassium dichromate (containing about 100 mg dichromate) to 100 ml milk (or the respective amounts to a bigger volume). Absolute accuracy is not important, as finally there should be an excess of dichromate in the milk (visible as yellow colour) which will be reduced to (green) Cr(III) before analysis.

If the milk is to be collected at home, the tablet may be placed in the collection jar before it is given to the donor. CAUTION: The mother must be told to keep the jar with potassium dichromate away from persons in the household, in particular children, as this is a toxic chemical.

4.4 Biosafety

One of the criteria for selecting women as potential donors is that both the mother and infant should be apparently healthy with a normal pregnancy. The reasons for this criterion are to avoid extra demands on a mother who is already experiencing difficulties and to minimize results that may be caused by medical conditions (for example, sudden loss of weight may alter the body burden of POPs and concentrations in human milk). Consequently, donors with previously diagnosed clinical hepatitis, malaria, AIDS and other such diseases should be excluded from the study. In many countries, pregnant women are screened for a number of infectious diseases so that their health status can be evaluated.

In countries which have established HIV screening of pregnant women, the National Coordinator should decide whether HIV-positive donors should be excluded from the study. In this regard, potential weight loss of 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. While 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 is 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 for countries with HIV morbidity and no HIV screening, human milk samples should be considered contaminated and heat-treated as above.

4.5 Preparation of Pooled Samples

To prepare pooled samples, the 50 individual milk samples are homogenized by shaking for 5 minutes. The 50 ml individual samples have to be split in two portions of 25 ml each (see Figure 4). 25 ml are taken as individual samples for analysis of the countries for analytically simple POPs and 25 ml are put into a 2000 ml bottle to prepare the pooled sample (50 x 12 ml = 1250 ml pooled sample). In this way it is guaranteed that all pooled samples supply the minimum required amount of 500 ml for analyses and 750 ml for the WHO Global Human Milk Bank.

Each individual and pooled sample is to be labelled with a unique identification code.

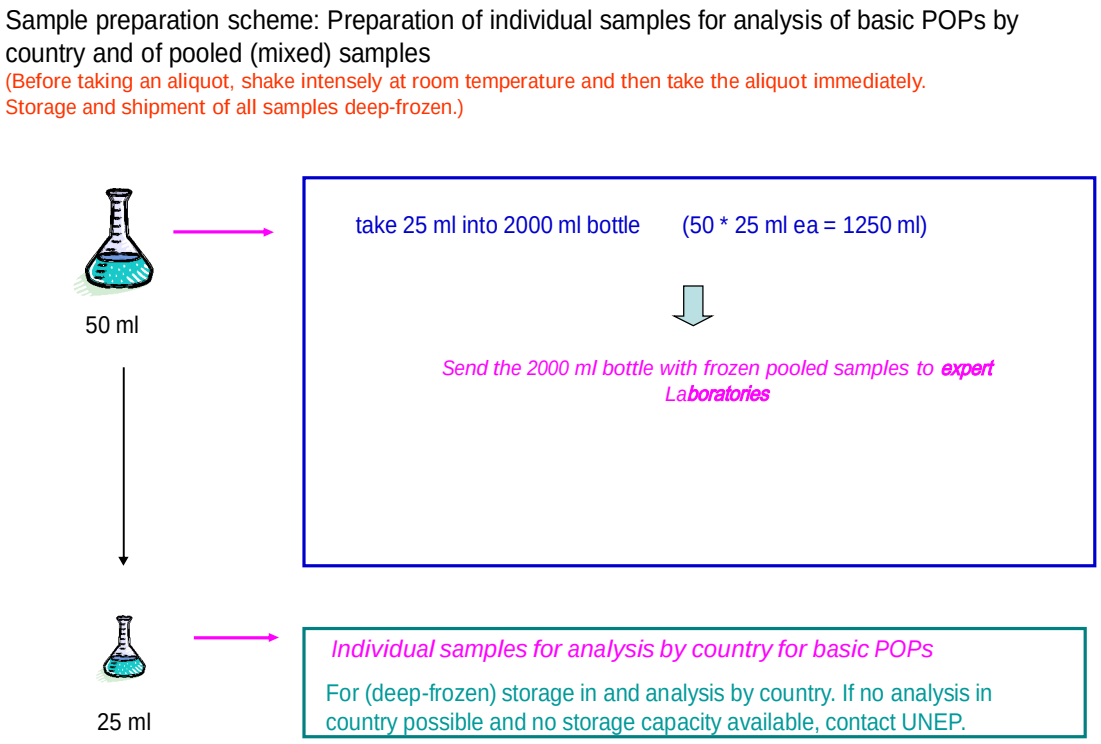


Figure 4: Sample preparation scheme

4.6 Transporting of Samples and Contact Data of Reference Laboratory

The individual samples containing 25 ml each should be sent to the (national) laboratory designated by the National Coordinator, the pooled sample to the expert Laboratory accompanied by the completed summary of information (see Annex 7).

The receiving laboratory shall notify when the package will be sent and its likely time of arrival. In particular before the long-range shipment of the samples to the expert Laboratory, the National Coordinator is urgently advised to contact this expert laboratory. The contact e-mail for CVUA Freiburg is pops@cvuafr.bwl.de; contact person for any organizational questions is Dr. Karin Malisch (karin.malisch@cvuafr.bwl.de). The frozen samples should be sent to the following address:

Dr. Rainer Malisch
Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg
(State Institute for Chemical and Veterinary Analysis of Food)
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 as possible. Samples should be frozen at -20°C, packed with cooling elements and sent to the destination. Countries should not use dry ice as dry ice is a hazardous good e.g. for DHL and TNT in Germany and will be only transported if these couriers have a contract with the sender. In countries where temperature control is not possible, the preservation of the sample should be maintained by the addition of 100 mg potassium dichromate per 250 ml of milk.

For shipment, the following customs declaration is necessary (see Annex 9). In this 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 has to be attached to the shipment

After the arrival of the pooled human milk samples the bottles will be kept at -20°C in a freezer.

The national or expert Laboratory should confirm receipt of the package.

5 ANALYSIS OF THE MOTHERS' MILK SAMPLES

Chapters 4.2.2 and 4.2.3 explain the general concept of sampling (collection of individual samples, preparation of pooled samples) and analysis.

5.1 Samples

The 50 individual samples should be analysed for pesticide POPs and marker PCBs at the laboratory selected by the National Coordinator. 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 reported on a fat basis. In this regard, the literature should be consulted.

Ideally this laboratory should be located in the country, but emphasis should be on analytical proficiency as demonstrated by adequate quality assurance procedures and confirmed by successful participation in inter-laboratory studies for pesticide POPs and marker PCBs. Based on past analytical quality assurance studies, some laboratories have had difficulty in qualifying for POPs proficiency. Note that this was true for laboratories in both developing and developed countries.

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

5.2 Analysis of Pooled Samples

The pooled sample are analysed by the State Institute for Chemical and Veterinary Analysis of Food (CVUA) in Freiburg, Germany, for the POPs listed in the Stockholm Convention (parent compounds and relevant transformation products) (see chapter 4.2.3, table 3)

This laboratory was appointed as the expert Laboratory for this study, having met all the requirements established by WHO at the Fourth Round for the Interlaboratory Calibration of PCDD, PCDF and PCB levels in human milk and having been selected as the EU Reference Laboratory for Dioxins and PCBs in Feed and Food and as the EU Reference Laboratory for Pesticides in Food of Animal Origin and Commodities with High Fat Content.

The expert Laboratory follows a rigorous quality control program to guarantee the accuracy and reliability of the results obtained in the study. The laboratory runs this program daily in the routine testing for PCDD, PCDF and PCB and POP agrotoxics, primarily on foodstuff samples.

Annexes

ANNEX 1: THE VALUE 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 needs 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 diarrhea in artificially fed infants was 14 times that of fully breastfed infants. Even in developed countries non-breastfed children have higher rates of diarrhea. 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, diarrhea 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 QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS

Questionnaire for Potential Human Milk Donors

UNEP-coordinated Survey of Human Milk for Persistent Organic Pollutants

CONFIDENTIAL!

Section 1: Personal Information

Name	Phone number	Today's Date (dd/mm/yyyy)
	e-mail	
Address		

Section for National Coordinator	
Individual Identification Code	Pool Identification Code

Based on established criteria, is the participant eligible?

Yes ☐ No ☐

What is the status of donor in regard to the survey?

Selected ☐ Reserve ☐ Not Selected ☐

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: Screening Questionnaire	
Name of Interviewer:	Date of interview (dd/mm/yyyy):
Place of interview:	
1. Are you planning to breastfeed your child? Yes ☐ No ☐	
2. Is this your first child? Yes ☐ No ☐	
3. Are you expecting a single child? (not twins) Yes ☐ No ☐	
4. Are you having a normal healthy pregnancy? Yes ☐ No ☐	
5. Have you lived in your current area for 10 years? Yes ☐ No ☐ * If no, actual number of years _____	
6. Are you under 30 years of age? Yes ☐ No ☐ * 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 ☐ No ☐	

***Note that if the answers to questions 5 or 6 was "no", please ask what the participant's actual residence time and/or birth date.**

Instruction to interviewer: If any answers to questions 1-6 were "no" or if the answer to question 7 was "yes", the participant is not eligible for this survey. Please thank the participant for their interest in the survey and end this interview. If all answers are "yes" except question 7, proceed with Section 3.

Section 3: Health History Questionnaire

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 last 10 years:

urban (city) ☐ rural (countryside) ☐

3. How would you describe your dietary habits before pregnancy?

Mixed diet ☐ Vegetarian but with milk and eggs ☐

Strictly vegetarian ☐ Other ☐

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?

Fish from the sea ☐ Freshwater fish ☐ Both ☐

Please state the species if known :

5. Was your mother born in this country?

Yes ☐ No ☐

6. Were you breastfed?

Yes ☐ No ☐ Do not know ☐

If you know, for how long? _____

7. Were you engaged in work other than housework before pregnancy?

Yes ☐ No ☐

If yes, please state the duration and describe type of work :

8. Has the inside of your house been sprayed with DDT in order to prevent mosquitoes?

Yes ☐ No ☐ Do not know ☐

If yes, when? _____

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 sample will be collected today, proceed to Section 4.

To be completed by the National Coordinator if using pre-selection option		
Mother's Name	Phone number Email	Date of delivery (dd/mm/yyyy)
Address		
Status of donor in regard to the survey Selected ☐ Reserve ☐		
Individual Identification Code		

Section 4. To be completed by the Sample Collector	
Name of Collector:	Date of sampling (dd/mm/yyyy):
Clinic of Collection:	Place of collection:

Postnatal Information (to be taken at the time of sampling)
1. Are you prepared to sign the consent form? Yes ☐ No ☐ If yes, attach signed consent form. If no, mother is not eligible to participate in survey.
2. How old is your infant? less than 3 weeks* ☐ 3-4 weeks ☐ 5-8 weeks ☐ more than 8 weeks** ☐
3. What is the sex of your infant? Male ☐ Female ☐
4. Is your current weight different than your weight before pregnancy? Gained ☐ Lost ☐ Not changed ☐
5. Can you provide a sample now? Yes ☐ Later ☐ When? _____ At home ☐ If you want to take the sample at home, do you have a refrigerator? Yes ☐ No ☐ ***

* Infant has to 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.
*** A tablet of potassium dichromate needs to be added to the collection jar and the mother caution about its potential toxicity.

ANNEX 3: SUMMARY INFORMATION ON HUMAN MILK SURVEYS JOINTLY ORGANIZED BY WHO AND UNEP[11]

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 signaled 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 have a capacity to accumulate in many fat-containing foods as well as the human body where traces of POPs can be found in human milk. The most commonly encountered POPs are organochlorine pesticides, such as DDT, industrial chemicals, most notably polychlorinated biphenyls (PCBs), 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 2004, 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.

Meeting under the auspices of the United Nations Environment Programme (UNEP), parties to the Convention have identified human milk as one of the core matrices to be monitored to evaluate the impact of the Stockholm Convention in reducing emissions of POPs. In conducting the surveys of POPs in human milk as joint WHO/UNEP programmes, monitoring of all 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.

[11] This information is provided for survey administrators and interested participants who wish to have more details on the survey, the Stockholm Convention on POPs and expected outcomes.

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 don't change very much 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 agreement. 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 well as be mixed with samples from at least 25 other mothers for analysis.

These results may also be combined with those of other countries to given 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. Information 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, but 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: EXAMPLE OF A PRO FORMA INVOICE FOR SHIPMENT OF GLASSWARE

Chemisches und Veterinäruntersuchungsamt Freiburg

World Health Organization

UNEP

WHO/UNEP Reference Laboratory

For determination of Persistent Organic Pollutants (POPs) in human milk

Chemisches und Veterinäruntersuchungsamt Freiburg
Postfach 100462 ◆ 79123 Freiburg

Freiburg,

Phone: 004917622178563

Contact: Dr. Karin Malisch

e-mail Karin.Malisch@cvuafr.bwl.de

Invoice No. 5477.11-16 012

Sender	WHO/UNEP-Reference Laboratory c/o State Institute for Chemical and Veterinary Analysis of Food Freiburg Karin Malisch
Address	Bissierstr. 5 D-79114 Freiburg
Phone No	+49 761 8855 109
Fax No	+49 761 855 100
Receiver	

No. Units	Name of Item	Description of Item	Country of Origin	Unit Value	Total Value
60	Glass bottles	100 ml (net weight ca. 0,2 kg)	Germany	€ 0,20	€ 12.00

Dienstgebäude der bearbeitenden Stelle:
Bissierstraße 5 Telefon: (07 61) 88 55-0
79114 Freiburg Telefax: (07 61) 88 55-100
Tram-Linie 3 (Haid): Haltestelle Bissierstraße

E-Mail:
poststelle@cvuafr.bwl.de

Sprechzeiten:
Mo-Do: 9^{00}-11^{45} Uhr
14^{00}-15^{30} Uhr
Fr: 9^{00}-12^{00} Uhr

Bankverbindung:
Landesoberkasse Baden-Württemberg,
Baden-Württembergische Bank
(BLZ 600 501 01), Kto. 746 95341 03

Chemisches und Veterinäruntersuchungsamt
Freiburg

1	Glass Bottle	2000 ml (net weight ca 1 kg) **<u>for scientific use only</u>**,	Germany	€ 4,00	€ 4,00

61 Units Totaling € 16

The glassware is for an international capacity building project on determination of Persistent Organic Pollutants (POPs) in human milk organized by United Nations Environment Programme (UNEP)

The glassware is provided by UNEP through the WHO/UNEP Reference Laboratory to the countries to collect the samples, prepare mixed (pooled) samples and send them back to the WHO Reference Laboratory in Germany.

I declare all of the information to be true to the best of my knowledge.
We certify that this invoice shows the full value of the goods and that no further invoice will be issued.

If you have any questions, please, contact us at the above mentioned address.

Sincerely,

Dr. Karin Malisch

ANNEX 6: 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 jar 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.
- You should avoid using ointments on your nipples before collecting your milk. If you have used ointment that day, you should wash your nipples with soap and 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 sample frozen if possible. The sample should be delivered to the place the National Coordinator has identified as soon as possible and protected from high temperatures during the transport. If refrigeration is not available in your home, your collection jar might contain a tablet of a chemical that will preserve your milk. However, you should collect your sample in one day and return it the clinic the next day. You should be careful to keep the jar containing the chemical out of the reach of children as it is dangerous if eaten.

ANNEX 7[12] SUMMARY INFORMATION FOR A POOLED SAMPLE

Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants

SUMMARY INFORMATION FOR A POOLED SAMPLE
(Based on confidential questionnaires from mothers donating human milk samples)

Country	Pool Identification code	Number of mothers in the pool

1.Ages of the mothers

Mean

Range

2.Mother's height (in cm)

Mean

Range

3.Mother's weight before pregnancy

Mean (in kg)

Range (in kg)

4. Child's age in weeks at sampling

Mean

Range

5. Area of residence during last 10 years: (% of the total mothers of the pool)

Urban ☐ rural ☐

6. Mother's **dietary habits** (% of total mothers in the pool)

Mixed diet ☐ Vegetarian but with milk and egg ☐

Strictly vegetarian ☐ Other ☐

[12] Source: WHO, Fourth WHO-coordinated survey of human milk for persistent organic polllutans in cooperation with UNEP, Guidelines for Developing a National Protocol, revised 1 Oct 2007

7. Mother born in the country
(% of total mothers in the pool)

8. Mother raised by breastfeeding
(% of total mothers in the pool)

9. Mother's mother born in the country
(% of total mothers in the pool)

10. Mothers working before pregnancy
(% of total mothers in the pool)

11. Exposure to DDT from inside house spraying in order to prevent mosquitoes
(% of total mothers in the pool)

12. Mothers whose current weight that is less than their weight before pregnancy (% of total mothers in the pool)

13. Mother's consumption of food (% of mother in the pool)

	Fish	Marine Mammals	Seafood other than fish and mammals	Milk and milk products	Meat and poultry	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 mother consumed most often (% of the mother in the pool)

Fish from the sea Fresh fish Both

15. POPs analyses requested besides the twelve (12) Stockholm POPs: None _____ List _____		
Date (dd/mm/yyyy)	Name of National Coordinator	Signature

ANNEX 8: CUSTOMS DECLARATION FOR SHIPMENT OF HUMAN MILK SAMPLES

Chemisches und Veterinäruntersuchungsamt Freiburg

World Health Organization

UNEP

WHO/UNEP Reference Laboratory

For determination of Persistent Organic Pollutants (POPs) in human milk

Chemisches und Veterinäruntersuchungsamt Freiburg
Postfach 100462 ◆ 79123 Freiburg

Freiburg,

Phone: 0049-761-8855-133
Contact: Dr. Karin Malisch
e-mail karin.malisch@cvuafr.bwl.de

Declaration

This package contains breast milk samples for an international study on determination of Persistent Organic Pollutants (POPs) in human milk organized by the World Health Organisation (WHO) in cooperation with United Nations Environment Programme (UNEP).

The country xxx collected the breast milk samples.

1. **The human breast milk samples are <u>not infectious.</u>**
2. **The human breast milk samples do <u>not have a commercial value.</u>**
3. **The human breast milk samples are shipped to the WHO/UNEP Reference Laboratory in Germany <u>only for analytical reasons</u>.**
4. **The human breast milk samples will <u>not be used for any kind of application for humans or animals or for any kind of pharmaceutical reasons.</u>**
5. **The samples are of <u>no commercial value</u>**

If you have any questions, please, contact us at the above mentioned address.

Sincerely,

Dr. Karin Malisch

Dienstgebäude der bearbeitenden Stelle:
Bissierstraße 5 Telefon: (07 61) 88 55-0
79114 Freiburg Telefax: (07 61) 88 55-100
Tram-Linie 3 (Haid): Haltestelle Bissierstraße

E-Mail:
poststelle@cvuafr.bwl.de

Sprechzeiten:
Mo-Do: 9^{00}-11^{45} Uhr
14^{00}-15^{30} Uhr
Fr: 9^{00}-12^{00} Uhr

Bankverbindung:
Landesoberkasse Baden-Württemberg,
Baden-Württembergische Bank
(BLZ 600 501 01), Kto. 746 95341 03