



**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)**

---

WHO Library Cataloguing-in-Publication Data

Fourth WHO-Coordinated survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP -Guidelines for Developing a National Protocol

1.Human exposure 2.Persistant Organic Pollutants 3.Stockholm Convention – evaluation  
4.Human milk I.World Health Organization.

ISBN XXXXXX

(LC/NLM classification: QT 235)

**© World Health Organization 2007**

All rights reserved. Publications of the World Health Organization can be obtained from Marketing and Dissemination, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 2476; fax: +41 22 791 4857; e-mail: [bookorders@who.int](mailto:bookorders@who.int)). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to Publications, at the above address (fax: +41 22 791 4806; e-mail: [permissions@who.int](mailto:permissions@who.int)).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The World Health Organization does not warrant that the information contained in this publication is complete and correct and shall not be liable for any damages incurred as a result of its use.

**Food Safety, Foodborne Diseases and Zoonoses Department**  
**World Health Organization**  
**Geneva, Switzerland**  
E-mail: [foodsafety@who.int](mailto:foodsafety@who.int)  
Fax: +41 22 791 4807  
<http://www.who.int/foodsafety>

# **Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP**

## **Guidelines for Developing a National Protocol**

### **Summary**

Since 1976 the World Health Organization through its GEMS/Food Programme has collected and evaluated information on levels of persistent organic pollutants in foods, including human milk. Over the period 1987-2003, it has coordinated three international studies of human milk to assess the levels and trends of polychlorinated dibenzodioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls. Analysis of human milk, maternal blood and adipose tissue are all relevant matrices for assessment of body burdens for persistent organic pollutants. However, human milk is recognized as the preferred matrix because has several important advantages. Biomonitoring of human milk data can provide information on the exposure of the mother as well as the infants. Furthermore, such information provide guidance on the need for measures to reduce levels of this substances in food, which is the main source of exposures for most people. More recently, it has been recognized that human milk is an ideal matrix to generally monitor levels of persistent organic pollutants in the environment.

In 2004, the Stockholm Convention on Persistent Organic Pollutants was ratified by governments to decrease environmental and human exposure to twelve priority substances in this class. The revised WHO guidelines for developing a national protocol describe the basic study design that can be used to monitor human exposure over time in order to, among other things, see if the Stockholm agreement is actually effective in reducing the release of these chemicals into the environment. These guidelines continue to support the monitoring of persistent organic contaminants for human health and food-chain contamination purposes. The protocol guidelines were designed based on the advice of experts in the field and on extensive experience of certain countries in undertaking similar surveys using human samples, including human milk. In order to promote reliability and comparability, participating countries are encouraged to adhere as closely to this protocol as possible. Ethical issues, including informed consent of donors and confidentiality, are major considerations in this protocol. Given that breastfeeding reduces child mortality and has health benefits that extend into adulthood, every effort has been made to protect, promote and support breastfeeding in the context of these studies.

## Contents

Summary	i
<b>1. Background</b>	<b>1</b>
<b>2. Aims of the protocol</b>	<b>2</b>
<b>3. General principles</b>	<b>3</b>
<b>4. Developing a national protocol</b>	<b>3</b>
4.1 Pooled versus individual samples	4
4.2 Number of samples	5
4.3 Selection of donors	5
4.4 Interviewing potential donors	7
4.5 Criteria and selection of participants	7
4.6 Collection of samples	8
4.7 Biosafety	9
4.8 Transporting of samples	9
4.9 Preparation of individual and pooled samples	9
4.10 Analysis of individual samples and capacity building	10
4.11 Analysis of pooled samples	10
4.12 Data handling and assessment considerations	11
<b>5. Ethics</b>	<b>11</b>
<b>6. Financial Aspects</b>	<b>12</b>
<b>7. Publication of results</b>	<b>12</b>
<b>8. Coordination of the survey</b>	<b>12</b>
<b>9. Additional references and reading</b>	<b>12</b>
<b>ANNEX 1 THE VALUE OF BREASTFEEDING</b>	<b>13</b>
<b>ANNEX 2 LIST OF PERSISTENT ORGANIC POLLUTANTS</b>	<b>14</b>
<b>ANNEX 3 STATISTICAL CONSIDERATIONS IN PROTOCOL DESIGN</b>	<b>18</b>
<b>ANNEX 4 QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS</b>	<b>25</b>
<b>ANNEX 5 SUMMARY INFORMATION ON THE WHO HUMAN MILK SURVEY</b>	<b>30</b>
<b>ANNEX 6 MODEL INFORMED CONSENT FORM</b>	<b>31</b>
<b>ANNEX 7 GUIDANCE FOR MOTHERS COLLECTING MILK SAMPLES AT HOME</b>	<b>33</b>
<b>ANNEX 8 SUMMARY INFORMATION FOR A POOLED SAMPLE</b>	<b>35</b>
<b>ANNEX 9 ESTIMATED TIMELINE AND BUDGET</b>	<b>37</b>
<b>ANNEX 10 ADDITIONAL REFERENCES AND READING</b>	<b>39</b>
<b>ANNEX 11 WHO TEF Value</b>	<b>40</b>

## 1. Background

Persistent organic pollutants (POPs) are a group of chemicals which have been intentionally or inadvertently produced and introduced into the environment. Due to their stability and transport properties, they are now widely distributed around the world, and are even found in places where they had never been used, such as the arctic regions. Given their long half-lives and fat solubility, POPs tend to bioaccumulate in animals, particularly in long-lived species at the top of the food-chain. POPs appear at higher concentrations in fat-containing foods, including fish, meat, eggs and milk. POPs are also present in the human body and traces can be found in human milk. The most commonly mentioned POPs are organochlorine pesticides, such as DDT, industrial chemicals, most notably polychlorinated biphenyls (PCBs), and industrial by-products, especially polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). As a group, POPs are of concern for both environmental and human health concerns, most notably, because of their potential effects on the endocrine system.

Data on certain POPs in food, including human milk, have been collected and collated by the World Health Organization (WHO) GEMS/Food Programme<sup>1</sup> for over 25 years. In 1998 GEMS/Food published a health assessment of certain organochlorine contaminants in human milk<sup>2</sup>. In addition, WHO has coordinated three special surveys of PCDDs, PCDFs and dioxin-like PCBs in human milk covering the periods 1987-1988, 1992-1993 and 2000-2003. To ensure reliability and improve comparability, WHO has routinely carried out inter-laboratory analytical quality assurance studies of POPs. For the third round of WHO-coordinated exposure studies, the State Laboratory for Chemical and Veterinary Analysis of Food (CVUA) in Freiburg, Germany qualified as the WHO Reference Laboratory for POPs in Human Milk based on stringent pre-agreed criteria<sup>3</sup>.

With the ratification of the Stockholm Convention on POPs in early 2004, the international community signalled its commitment to reduce or eliminate production and emission of twelve important POPs<sup>4</sup> into the environment and ultimately, the human body. Of particular relevance to WHO, Article 7 of the Convention requires each country<sup>5</sup> to develop a National Implementation Plan while Article 16 requires an effectiveness evaluation of the Convention four years after its ratification. In this regard, experts convened by the United Nations Environment Programme (UNEP) to consider the Global Monitoring Plan (GMP) for POPs have recommended that the monitoring of human milk be carried out in close collaboration with WHO<sup>6</sup>. In addition to these two sections, Article 11 of the treaty addresses research needs relevant to public health protection. In May 2004 the first Conference of Parties (COP) to the Stockholm Convention requested that field tests be carried out on a national or regional basis to assess the cost and feasibility of obtaining monitoring data from various matrices, including human milk. In 2005, GEMS/Food in collaboration with the CVUA laboratory initiated a pilot study of human milk,

---

<sup>1</sup> The Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) is now implemented by WHO in collaboration with its participating institutions located in over 120 countries around the world.

<sup>2</sup> These included DDT and metabolites, hexachlorobenzene, alpha-, beta- and gamma-hexachlorocyclohexane, aldrin and dieldrin and marker polychlorinated biphenyls.

<sup>3</sup> Inter-laboratory quality assessment of levels of PCDDs, PCDFs and dioxin-like PCBs in human milk and blood plasma, WHO Report EUR/00/5020352, WHO Regional Office for Europe, Copenhagen, 2000

<sup>4</sup> The twelve POPs presently included under the Convention are aldrin, DDT, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls, polychlorinated dibenzodioxins and polychlorinated dibenzofurans.

<sup>5</sup> Note that the term "country" is used in this guideline, but within the context of the Stockholm Convention, this is also assumed to include "parties".

<sup>6</sup> United Nations Environmental Programme (UNEP) Chemicals, Proceedings: UNEP Workshop to develop a POPs Global Monitoring Plan to support the effectiveness evaluation of the Stockholm Convention, Geneva, Switzerland, 24-27-March 2003

which confirmed the cost-effectiveness of measuring all twelve POPs presently covered under the Stockholm Convention in pooled human milk samples by the introduction of additional analytical steps. This study also included selected polybrominated diphenylethers (PBDEs), which are considered as possible candidates for future risk management. In May 2005, WHO and UNEP entered into a Memorandum of Agreement for the coordination of human milk surveys for the purpose of the Stockholm Convention.

At the same time, evidence for the health advantages of breastfeeding and scientific evidence to support breastfeeding has continued to increase. WHO can now say with full confidence that breastfeeding reduces child mortality and has health benefits that extend into adulthood. 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.<sup>7</sup>

The basic intent of this document is to provide guidance for countries that have ratified or plan to ratify the Stockholm Convention for constructing a national protocol for the monitoring of POPs in human milk. This document can also be used by countries that have taken part in previous WHO-coordinated exposure studies of human milk for POPs to improve and expand their existing monitoring efforts. This document was developed based on the advice and suggestions of the ad hoc WHO Human Milk Survey Advisory Group<sup>8</sup>, which most recently met in September 2006. Protocols consistent with these guidelines should serve the needs of the Stockholm Convention. However, it should also be borne in mind that POPs remain a public health concern and information on human exposure to POPs is essential for assessment and, where warranted, further management to protect human health.

## **2. Aims of the protocol**

The main aims of the planned survey are as follows:

To provide information on the public health implications of POPs by:

- extending and strengthening the WHO GEMS/Food studies of human exposure to include all Stockholm POPs;
- providing data to health, environment, agriculture and fisheries sectors on human exposure to POPs for possible use in risk assessment and management; and,
- identifying needs for further national studies, including epidemiological follow-up studies.

To provide accessible, reliable and comparable data on levels of POPs in human milk for purposes of the Stockholm Convention by:

- assisting in the formulation or revision of National Implementation Plans under Article 7;
- contributing to the evaluation of the effectiveness of Stockholm Convention in the reduction or elimination of the release of POPs into the environment as required under Article 16; and,
- addressing relevant provisions of Article 11 regarding research and monitoring of POPs.

Human milk surveys should support and strengthen, where feasible, national capabilities for the monitoring and sound management of POPs as well as other potentially hazardous chemicals in the

---

<sup>7</sup> WHO (2006) The International Code of Marketing of Breast-milk Substitutes. Frequently Asked Questions. Geneva, World Health Organization. ISBN 92 4 159429 2

<sup>8</sup> A list of current members of this group is available at <http://www.who.int/foodsafety/chem/pops/en/>

food supply. It is recognized that these surveys will cover a wide range of countries, some with large differences in food consumption patterns and environmental levels of POPs. However, these surveys are not primarily intended to compare levels of POPs among countries, but rather to examine levels within countries over time.

Through interaction with mothers before and after delivery, these surveys should have sufficient safeguards to avoid undermining breastfeeding. Breastfeeding should be protected, promoted and supported as the optimal way of feeding infants. Finally, these surveys should be seen as promoting breastfeeding as they will be instrumental in the ultimate elimination of POPs in the environment and thus in breast milk. This is consistent with the Global Strategy for Infant and Young Child Feeding, which was endorsed by the World Health Assembly and the Executive Board of UNICEF in 2002.

### **3. General principles**

As a general principle, all those involved in the application of these guidelines for developing a national protocol for monitoring human milk for POPs should be careful not to undermine current efforts by governments, the World Health Organization, UNICEF and other international organizations to promote exclusive breastfeeding below 6 months of age with continued breastfeeding up to two years. As a matter of principle, all persons involved in this survey, 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. Annex 1 provides a perspective on why breastfeeding is important, which should be required reading by all persons administering and participating in the survey.

In keeping with the intended aims to support Articles 7, 11 and 16 of the Stockholm Convention, these guidelines also conform to the general principles identified in the Guidance for Global Monitoring Programme for POPs<sup>9</sup> in regard to practicality, feasibility and sustainability. In this regard, these guidelines also place great emphasis on the need for demonstrated proficiency in analytical results by laboratories monitoring POPs, as was the case with previous WHO GEMS/Food surveys of human milk for PCDDs, PCDFs and dioxin-like PCBs.

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.

### **4. 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 as mentioned above, especially for generating comparable monitoring data over time. The National Coordinator should be responsible for the overall planning and implementation of the survey in the

---

<sup>9</sup> United Nations Environmental Programme (UNEP) Chemicals, Guidance for a Global Monitoring Programme for Persistent Organic Pollutants, 1<sup>st</sup> edition, June 2004

country assisted by appropriate health, laboratory and administrative staff. In particular, the National Coordinator should assure that the survey meets all national ethical requirements for human subjects. If the National Coordinator does decide to modify the national protocol, any changes should be fully documented and be adhered to from that time forward. Because deviations from these protocol guidelines may inadvertently result in reduced reliability or comparability, the National Coordinator may wish to seek advice from the ad hoc WHO Human Milk Survey Advisory Group when considering significant modifications.

In developing a national protocol, the National Coordinator will need to take into account the following considerations.

#### **4.1 Pooled versus individual samples**

In past WHO-coordinated exposure studies of PCDDs, PCDFs and dioxin-like PCBs, only pooled<sup>10</sup> 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. On the other hand, the analysis of individual samples can provide information on the distribution of exposures and on factors possibly contributing to exposure. Such data are also essential to statistically validate changes in levels of POPs over time. Therefore, these guidelines recommend the use of pooled samples to monitor levels of PCDDs, PCDFs and dioxin-like PCBs in human milk, while individual samples should be analysed for the basic pesticide POPs and marker PCBs. The latter can be determined by a method that uses gas chromatography/electron capture detector, which is basic instrumentation available in many developing countries. In this document, these two groups of POPs are referred to as *analytically simple POPs* and *analytically complex POPs*, respectively. Annex 2 provides a list of *analytically simple* and *complex POPs* covered by these guidelines, including related degradation products, which should be analysed and reported along with the parent compound. As an internal quality control check, pooled sample will also be analysed for *analytically simple POPs* because the average value from individual samples should be equal to the pooled sample value. In addition, other POPs not currently included in the Stockholm Convention may also be considered for analysis in the pooled and/or individual samples, depending on the national priorities and available resources. A list of optional POPs that may be included in the analysis of pooled samples is given in Annex 2.

As a first step, the National Coordinator has to define the specific aims and resources available. 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 levels 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.

It should be noted that these guidelines only address a survey with two sampling periods. The first sampling period will be conducted to determine baseline levels 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 four or five years later (or other time period deemed appropriate). Future samplings should be undertaken at regular intervals and could be

---

<sup>10</sup> A pooled sample is a composite made by mixing equal volumes of milk from individual samples.

incorporated in the protocol at this time. However, the monitoring of human milk for POPs should be considered a long-term activity.

#### **4.2 Number of samples**

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 levels of POPs over time. Annex 3 provides some statistical considerations in protocol design. However, a country-specific analysis can only be completed after information on distribution of POPs in individual samples becomes available. Consequently, this issue should be revisited prior to the implementation of the second sampling.

#### **4.3 Selection of donors**

The survey protocol should be developed to assess the levels 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 levels of POPs in those foods will mainly determine the levels 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. Some factors known to have such effects have been identified and are recommended as exclusion criteria for these protocol guidelines. However, for those countries that have participated in previous WHO-coordinated exposure studies in human milk, the collection of samples from the same high-exposure locations should be continued in order to allow for time-trend comparisons. Note that these samples should be kept separate from other samples. While countries are free to include such high-exposure groups in their protocols, it should be recognized that time-trends in POPs levels observed in these groups should not be used for effectiveness evaluation under the umbrella of the Stockholm Convention.

The criteria for selection of donors presented below are designed to reduce the variability in the individual samples due to factors that are known to have influence on the levels of POPs in human milk. Because the two collection periods for this survey may be only four or five years apart, the reduction in variability is of particular importance. On the other hand, overly stringent criteria in the selection of donors may give rise to an insufficient number of qualified donors. Consideration of available statistics on primiparae mothers and experiences from other studies involving mothers may be of assistance

when developing selection criteria for donors. However, a general starting point could include the following:

- Mother should be primiparae.
- Mother should be under 30 years of age<sup>11</sup>.
- 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 area for at least the previous 10 years.
- Mother should not reside in local areas where emissions of POPs are known or suspected to result in elevated levels of POPs in the local population.
- Mothers 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. Each has benefits and disadvantages, but the second is simplest and most efficient. 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 (see Annex 1). 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 4). 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 4.4 below. 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 4). 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 offers advantages for the second and subsequent sample collections.

---

<sup>11</sup> 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.

#### **4.4 Interviewing potential donors**

The model questionnaire for donors (see Annex 4) 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 Section 2. If necessary, these criteria should be modified to balance the need for a suitable pool of possible donors with the wish to reduce factors<sup>12</sup> that contribute to variability. For selection before giving birth, initial interviews should be conducted at prenatal clinics at least 2 months prior to delivery. Interviewers should be familiar with the information on the specific benefits of breastfeeding contained in Annex 1 as well as the purpose and procedures of the survey as described in the document *Summary Information for WHO Human Milk Survey* (see Annex 5). Interviewers should also be aware of prenatal information on breastfeeding<sup>13</sup> 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 as presented in Annex 1. 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.

If the woman 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 5). 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 (see Annex 4) 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.

#### **4.5 Criteria and selection of participants**

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<sup>14</sup>. 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

---

<sup>12</sup> Harris CA, Woolridge MW and Hay AW (2001) Factors affecting the transfer of organochlorine pesticide residues to breastmilk. *Chemosphere*, 43:243-56.

<sup>13</sup> Prenatal information as defined under step 3 of the WHO/UNICEF BFHI programme and in the related training course session 3 (pages 52 – 70).

<sup>14</sup> A list of National POPs Contact Points and National Implementation Plans are available at the Stockholm Convention Secretariat Website at <http://www.pops.int/>

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. If necessary, the National Coordinator may consult with the ad hoc WHO Human Milk Survey Advisory Group for advice.

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.

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 and cost-effective method for the identification of donors.

#### **4.6 Collection of samples**

Once breastfeeding is well-established, sampling can be carried out between 3 to 8 weeks (21 days to 2 months) after delivery. At the time of sample collection, individual interviews should be used to complete the remaining information in Section 4 of the participant questionnaire (see Annex 4). Donors should already have also received verbal and written information concerning the survey (see Annex 5). The procedures of the survey should be explained, particularly the rights of the donor to withdraw from the survey without prejudice (see Annex 6 for a model). Following this, the donor should be requested to give their written consent on a standard *Informed Consent Form*. 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<sup>15</sup> after a feeding or while infant is nursing on the other breast, to take advantage of the let-down reflex of the mother. Depending on the preference of the mother and local customs, a human milk pump to facilitate expression can be provided. 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 7). 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.

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. If refrigeration is not available, a small tablet of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) may be added to chemically sterilize the milk.<sup>16</sup> If the milk is to be collected at home, the tablet may be placed in the

---

<sup>15</sup> Information to teach hand expression for collecting the milk can be found in different WHO/UNICEF materials such as the HIV and infant feeding Counselling Tools: Counselling Cards (Card 13) and Take-home Flyers. Available at: [http://www.who.int/child-adolescent-health/publications/NUTRITION/HIV\\_IF\\_CT.htm](http://www.who.int/child-adolescent-health/publications/NUTRITION/HIV_IF_CT.htm)

<sup>16</sup> 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.

collection jar before it is given to the donor. CAUTION: The mother must be told to keep the jar with potassium dichromate away from other children in the household as this is a toxic chemical.

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 GEMS/Food (see Annex 8). The National Coordinator should also provide to GEMS/Food copies of completed questionnaires without personal identification, i.e. without Section 1.

#### **4.7 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 levels 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.

#### **4.8 Transporting of samples**

After collection, the 50 samples containing 50 ml each should be sent to the laboratory designated by the National Coordinator. Shipping of the samples should be carried out by commercial carriers or other means in the most expeditious manner as possible. Samples should be frozen at -20 °C, packaged in dry ice and sent to the destination. 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. Each individual and pooled sample should be labelled with a unique identification code. Pooled samples should be sent to the WHO Reference Laboratory accompanied by the completed summary of information (see Annex 8). The receiving laboratory should be notified when the package will be sent and its likely time of arrival. The laboratory should confirm receipt of the package.

#### **4.9 Preparation of individual and pooled samples**

Qualified personnel should be available to undertake the sample handling to ensure sample integrity. The individual milk samples should be homogenized by heating to 38 °C and shaking for 10 minutes. The laboratory should then prepare individual and pooled samples. The individual samples should be comprised of 25 ml of human milk to be used for the analysis of *analytically simple POPs*, i.e. pesticide POPs and marker PCBs. For the pooled sample, 10 ml should be taken from each of the 50

individual samples to make one pooled sample of 500 ml. Of this 500 ml, 50 ml should be kept and used for the analysis of simple POPs by participating countries, and the remaining pooled sample of 450 ml will be analysed by WHO Reference Laboratory for both *analytically simple and complex POPs*, i.e. PCDDs, PCDFs and dioxin-like PCBs. The remainder of the collected sample of 15 ml should be pooled to form a 750 ml sample that will be sent to WHO for its Global Human Milk Bank. The Bank will be used in the future in case new POPs are added to the Stockholm Convention and for other scientific purposes. For countries having adequate resources, more than one pooled sample per 50 individual samples may be considered, e.g. one pooled sample for 25 individual samples. However, all pooled samples should supply the required amount of 500 ml per sample. If countries wish to analyse aliquots of the pooled samples for other contaminants, a modified protocol to collect more samples (from more individuals or higher sample amounts) will be required. The WHO Reference Laboratory can be consulted for further information.

#### **4.10 Analysis of individual samples and capacity building**

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 levels 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 or the ad hoc WHO Human Milk Survey Advisory Group may be able to provide advice.

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. In selecting a laboratory, National Coordinators might consult the list of GEMS/Food Participating Institutions<sup>17</sup>.

Based on past GEMS/Food 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. Therefore, before a contract is signed, the National Coordinator should request GEMS/Food to provide the candidate laboratory with a check sample from the WHO Reference Laboratory. The proficiency test will be provided at no cost. In addition, the laboratory should analyse an aliquot of the pooled sample in order to compare results with the WHO Reference Laboratory. Adequate determination of analytically simple POPs in the check sample provides an independent assessment of performance or will identify areas for possible capacity building. Sufficient amount of this check sample will be provided to allow its use also as a quality control sample when analysing the individual samples. Note that mean results of individual analyses can also be compared with the result of the pooled sample analysed by the WHO Reference Laboratory.

#### **4.11 Analysis of pooled samples**

The 450 ml pooled sample will be analysed for *analytically simple and complex POPs*, including PCDDs, PCDFs and dioxin like PCBs. In addition, polybrominated diphenylethers (PBDEs), polybrominated dibenzo-p-dioxins and dibenzofurans (PBrDDs/PBDFs) and mixed halogenated dibenzo-p-dioxins and dibenzofurans (PXDDs/PXDFs) may be included as options for determination in pooled samples if a more complete picture is desired. A complete list of *analytically simple and complex POPs* as well as some optional POPs is given in Annex 2.

---

<sup>17</sup> See [http://www.who.int/foodsafety/chem/GEMS\\_countries.pdf](http://www.who.int/foodsafety/chem/GEMS_countries.pdf)

The 450 ml pooled sample will be analysed by the State Institute for Chemical and Veterinary Analysis of Food (CVUA) in Freiburg, Germany, in accordance with the request of the National Coordinators. CVUA is the WHO Reference Laboratory for the fourth WHO-coordinated human milk study for POPs. All analytical results will be reported on a fat basis. The contact email for CVUA is [pops@cvuafr.bwl.de](mailto:pops@cvuafr.bwl.de)

#### 4.12 Data handling and assessment considerations

WHO GEMS/Food will maintain databases for all reporting requirements, including raw data from laboratories of both *analytically simple and complex POPs* and relevant data from donor questionnaires. This will ensure that the entire process, from sampling through to reporting concentrations, will be independently evaluated. For some POPs, results may be below the limit of determination. In such cases, the calculation of mean values from a set of individual data can be influenced by the values assigned to these results. In most cases, one-half the limit of determination should be used, but if the number of such results exceeds 60% of the total number of results, other procedures should be used, such as giving maximum and minimum values.<sup>18</sup>

All results should be reported in a format that is compatible with the GEMS/Food data structure for individual contaminants in food (see [www.who.int/foodsafety/publications/chem/gems\\_instructions/en](http://www.who.int/foodsafety/publications/chem/gems_instructions/en)). Data on levels of PCDDs, PCDFs and dioxin-like PCBs should be reported as individual congeners and in toxic equivalents using WHO toxic equivalence factors (WHO TEFs)<sup>19</sup>. Dissemination of results in aggregate form can be made through the WHO SIGHT (Summary of Information on Global Health Trends) portal. Release of other data will be made with the approval of the ad hoc WHO Human Milk Survey Advisory Group and the agreement of relevant National Coordinators.

In regard to national assessments, National Coordinators should also understand the uncertainty and variability in levels of POPs and in time trends. When making comparisons between countries, these data should be seen as indicating relative levels and trends rather than absolute differences in POPs. When possible, trends and other evaluations should include qualifying information, such as uncertainty and variability, to allow greater insight into data reliability and comparability

### 5. Ethics

Mothers donating samples of their milk should be informed of the nature and purpose of the survey and asked to sign an informed consent form for this purpose (see Annexes 5 and 6). These guidelines for developing a national protocol have been initially evaluated by the WHO Research Ethics Review Committee. However, it is the responsibility of the National Coordinator to ensure that the national protocol that is finally adopted meets all national ethical and informed consent requirements. The results of this survey are expected to strengthen the factual basis for the health risk assessment for infants and children and to promote environmental and other measures likely to reduce the concentrations of these chemicals in human milk.

Based on national requirements, National Coordinators should decide whether to provide donors with the results of individual and/or pooled samples. If such information is provided, considerable

---

<sup>18</sup> Reliable evaluation of low-level contamination of food - workshop in the frame of GEMS/Food-EURO. Kulmbach, Germany, 26-27 May 1995. See [http://www.who.int/foodsafety/publications/chem/lowlevel\\_may1995/en/](http://www.who.int/foodsafety/publications/chem/lowlevel_may1995/en/)

<sup>19</sup> The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds, *Martin van den Berg, et al.*, *Tox Sci Advance*, 2006. See [http://www.who.int/ipcs/assessment/tef\\_update/en/](http://www.who.int/ipcs/assessment/tef_update/en/) Note that in assessing time trends either the 1998 or 2005 TEFs should be used, but not both.

judgement must be used in drawing conclusions concerning levels of POPs. The provision of individual results should always be accompanied by an explanation giving the range of other results and a short interpretation of the health significance of the values. Once sufficient data are available, WHO will develop appropriate risk communication advice on this matter. In all cases, breastfeeding should be promoted as the best feeding mode for infants.

## **6. Financial Aspects**

Countries will be responsible for managing all funds necessary for conducting their national surveys, especially the costs associated with collection of individual samples, sample preparation and analysis, including handling and shipping. Countries will also be responsible for the preparation, and analysis of pooled samples. Countries should provide adequate facilities and other in-kind contributions to facilitate the collection, preparation and handling of the samples. An estimated timeline and budget are given in Annex 9.

WHO will be responsible for the costs for the overall management of the survey and maintaining data generated by this survey, including analytical results and information gathered through the questionnaire. The costs of meetings of the ad hoc WHO Human Milk Survey Advisory Group as well as any publication costs will also be covered by WHO. WHO will also support the cost of the proficiency testing scheme for pesticide POPs and marker PCBs.

## **7. Publication of results**

Results from participating countries will be collected and evaluated by WHO in accordance with the advice and guidance of the ad hoc WHO Human Milk Survey Coordinating Group. The results will be sent to all National Coordinators before being shared with the UNEP Stockholm Convention Secretariat. In addition, each country would be free to publish its own results.

## **8. Coordination of the survey**

The WHO Secretariat for this survey is located in the Food Safety, Foodborne Diseases Department, World Health Organization, Geneva, Switzerland. Contact information for the WHO Secretariat is provided below. WHO also operates six Regional Offices and WHO Representatives are present in most developing countries. These offices may also be contacted if further information is required.

WHO Secretariat will work with the National Coordinators identified for each country as well as with the Stockholm Convention Secretariat at UNEP Chemicals to assure that reports are timely and meet the needs of the Convention. WHO will consult the ad hoc WHO Human Milk Survey Advisory Group as necessary.

Secretariat for the Fourth WHO-Coordinated Survey of Human Milk for POPs

E-mail: [popsmilk@who.int](mailto:popsmilk@who.int)

Tel: +41 22 791 3557

Fax: +41 22 791 4807

Website: <http://www.who.int/foodsafety/chem/pops/en>

## **9. Additional references and reading**

Annex 10 provides a list of additional publications which may be of interest to National Coordinators in the preparation of their national survey protocols.

## THE VALUE OF BREASTFEEDING<sup>20</sup>

Breastfeeding is 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 diarrhoeal 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 6 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 2 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.

**Family planning/child spacing:** Breastfeeding delays the return of a woman's fertility. A woman who does not breastfeed is at increased risk of becoming pregnant again as early as six weeks after the birth of the child. All women, especially women who do not breastfeed, should have access to contraceptives within six weeks of delivery, if they so desire, to ensure the recommended interval between births. (A woman who exclusively, or nearly exclusively, breastfeeds during the first six months, and who remains amenorrhoeic [her menses, or periods, have not returned], has less than a 2% risk of becoming pregnant.)

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

---

<sup>20</sup> The National Coordinator may simplify this information depending on the needs and educational status of women involved in the survey.

## LIST OF PERSISTENT ORGANIC POLLUTANTS

### Analytically simple POPs - Pesticide POPs and Marker PCBs

#### **Aldrin**

#### **Chlordane** (total)

alpha-chlordane  
gamma-chlordane  
oxy-chlordane  
trans-nonachlor

#### **Dieldrin**

#### **DDT** (total)

o,p'-DDD  
p,p'-DDD  
o,p'-DDE  
p,p'-DDE  
o,p'-DDT  
p,p'-DDT

#### **Endrin** (total)

Endrin  
Endrin ketone

#### **Heptachlor** (total)

Heptachlor  
Heptachlor epoxide

#### **Hexachlorobenzene**

#### **Hexachlorocyclohexane (HCH)** (total)\*

Alpha-HCH  
Beta-HCH  
Gamma-HCH

#### **Mirex**

#### **Toxaphene** (total)

Parlar 26  
Parlar 50  
Parlar 62

#### **Polychlorinated biphenyls (PCBs)**

##### **Marker PCBs**

IUPAC No. 28  
IUPAC No. 52  
IUPAC No. 101  
IUPAC No. 138  
IUPAC No. 153  
IUPAC No. 180

\* Although hexachlorocyclohexanes are not currently among the 12 Stockholm Convention POPs, they are included here as they can be analysed with the analytically simple POPs and they may be considered candidates for inclusion in the treaty in the future.

**Analytically Complex POPs – PCDDs, PCDFs and dioxin-like PCBs**

**Polychlorinated dibenzodioxins (PCDDs) (total expressed in WHO TEFs)**

2,3,7,8-TCDD  
1,2,3,7,8-PeCDD  
1,2,3,4,7,8-HxCDD  
1,2,3,6,7,8-HxCDD  
1,2,3,7,8,9-HxCDD  
1,2,3,4,6,7,8-HpCDD  
1,2,3,4,6,7,8,9-OCDD

**Polychlorinated dibenzofurans (PCDFs) (total expressed in WHO TEFs)**

2,3,7,8-TCDF  
1,2,3,7,8-PeCDF  
2,3,4,7,8-PeCDF  
1,2,3,4,7,8-HxCDF  
1,2,3,6,7,8-HxCDF  
1,2,3,7,8,9-HxCDF  
2,3,4,6,7,8-HxCDF  
1,2,3,4,6,7,8-HpCDF  
1,2,3,4,7,8,9-HpCDF  
1,2,3,4,6,7,8,9-OCDF

**Dioxin-like polychlorinated biphenyls (PCBs) (total expressed in WHO TEFs)**

**Mono-ortho PCBs**

IUPAC No. 105  
IUPAC No. 114  
IUPAC No. 118  
IUPAC No. 123  
IUPAC No. 156  
IUPAC No. 157  
IUPAC No. 167  
IUPAC No. 189

**Non-ortho PCBs**

IUPAC No. 77  
IUPAC No. 81  
IUPAC No. 126  
IUPAC No. 169

## **Optional POPs for Pooled Samples**

### **Polybrominated diphenylethers (PBDEs) (total)**

2,2',4-tribromodiphenyl ether (BDE 17)  
2,4,4'-tribromodiphenyl ether (BDE 28)  
2,2',4,4'-tetrabromodiphenyl ether (BDE 47)  
2,3',4,4'-tetrabromodiphenyl ether (BDE 66)  
2,2',,4,4',5-pentabromodiphenyl ether (BDE 99)  
2,2',4,4',6-pentabromodiphenyl ether (BDE 100)  
2,2',3,4,4',5'-hexabromodiphenyl ether (BDE 138)  
2,2',4,4',5,5'-hexabromodiphenyl ether (BDE 153)  
2,2',4,4',5,6'-hexabromodiphenyl ether (BDE 154)  
2, 2',3,4,4',5',6-heptabromodiphenyl ether (BDE 183)  
2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE 209)

### **Hexabromocyclododecane (HBCDs)**

### **Polybrominated dibenzo-p-dioxins and dibenzofurans (PBDDs/PBDFs)**

### **Mixed halogenated (polybrominated/-chlorinated) dioxins and dibenzofurans (PXDDs/PXDFs)**

## STATISTICAL CONSIDERATIONS IN PROTOCOL DESIGN

### Introduction

The purpose of this annex is to help ensure that the results of the proposed survey to measure the levels of POPs in human milk and their evolution over time are scientifically valid in the light of the inherent uncertainty and variability anticipated. As the results are likely to be the basis for an evaluation of effectiveness of countries in the context of the Stockholm Convention on POPs, the survey design and statistical analysis must be carefully considered as part of the protocol design. This annex provides initial guidance to the National Coordinator on the statistical aspects of the survey. The National Coordinator is urged to seek statistical advice. In this regard, the ad hoc WHO Human Milk Survey Advisory Group may be in a position to provide basic advice when requested.

Succinctly stated, the aim of the survey is to identify significant evolutions in the levels of POPs between the base-line measurement and the follow-up measurement about four or five years thereafter. In order to make this determination, a statistical test must be met. The requirements of a statistical test are many, but most importantly, the samples must be random and the statistical distribution of the samples must be known. The precision of a statistical test depends on (a) the magnitude of the evolution (change between base-line and follow-up) to be detected, (b) the significance level of the test (explained later), (c) the number of samples upon which the test is constructed, and (d) the variance of the sample population.

As we are dealing with a poorly understood phenomenon, that is, the statistical distribution of POPs is often unknown. Therefore, much of the base-line measurements should be devoted to the study of the statistical properties of the various POPs. We expect different POPs to have different distributions, some perhaps so unwieldy so as to defy parametric statistical analysis and requiring non-parametric techniques. As a general rule, we wish to study the variance of the distributions in various strata of the target population, such as habitat, diet, age, and others, to be able to focus on the strata yielding smaller variances during the follow-up study.

To be able to respond to these requirements the samples must be selected by the National Coordinator with great care. The National Coordinator should directly manage the register of potential donors. Inclusion in the register is largely self-selection by the potential donor, but donors susceptible in skewing the samples by, for example, residing in highly contaminated areas, should be excluded. Information provided by potential donors should allow the register to be subdivided into strata as noted above. The National Coordinator, in consultation with the statistical consultant if possible, must ensure that the strata of interest are sufficiently represented in the register. When the register is completed, the National Coordinator should draw a random sample from the register of donors. The statistical consultant should be contacted to obtain a random number generator and instruction in its use in this context. Note that for donors selected after giving birth, a statistical analysis of the results may suggest changes in the criteria and number of donors in the second survey.

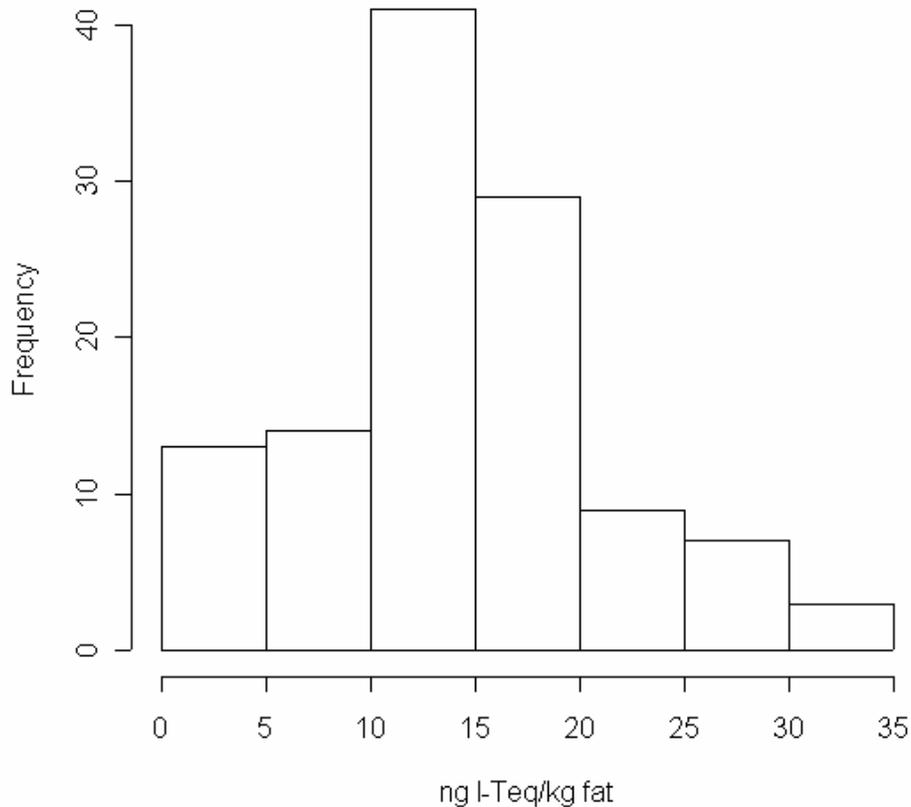
### Simulation of Statistical Analyses

To give the National Coordinator an intuitive appreciation of the statistical considerations involved, a simulation of the required analyses is presented. This simulation is particularly relevant to the study of *analytically simple POPs* as it assumes that the statistical analysis of contaminants should be based on individual, rather than pooled, samples. A cross-sectional study design is adopted, where one cohort is

sampled at base-line and another similar cohort is sampled at follow-up. As little on the distribution of POPs is known in most countries, we take as a sample dataset inferred data from a report<sup>21</sup> on PCDDs and PCDFs in human milk (expressed as I-TEQs)<sup>22</sup>. Figure 1 shows a histogram of these data, which appear to come from a normal distribution with a mean of approximately 14 ng per kg of fat and a standard deviation of approximately 6 ng per kg of fat. Indeed, Figure 2 shows the cumulative data fitted to a normal distribution with mean and variance cited above; the fit is obviously very close and there is no significant (statistical) difference between the dataset and the assumed normal distribution. This illustrates the first step in statistical analysis: determine the distribution of the data. Note that this approach can only be applied when individual results are available as will be the cases of pesticide POPs and marker PCBs.

**Figure 1**

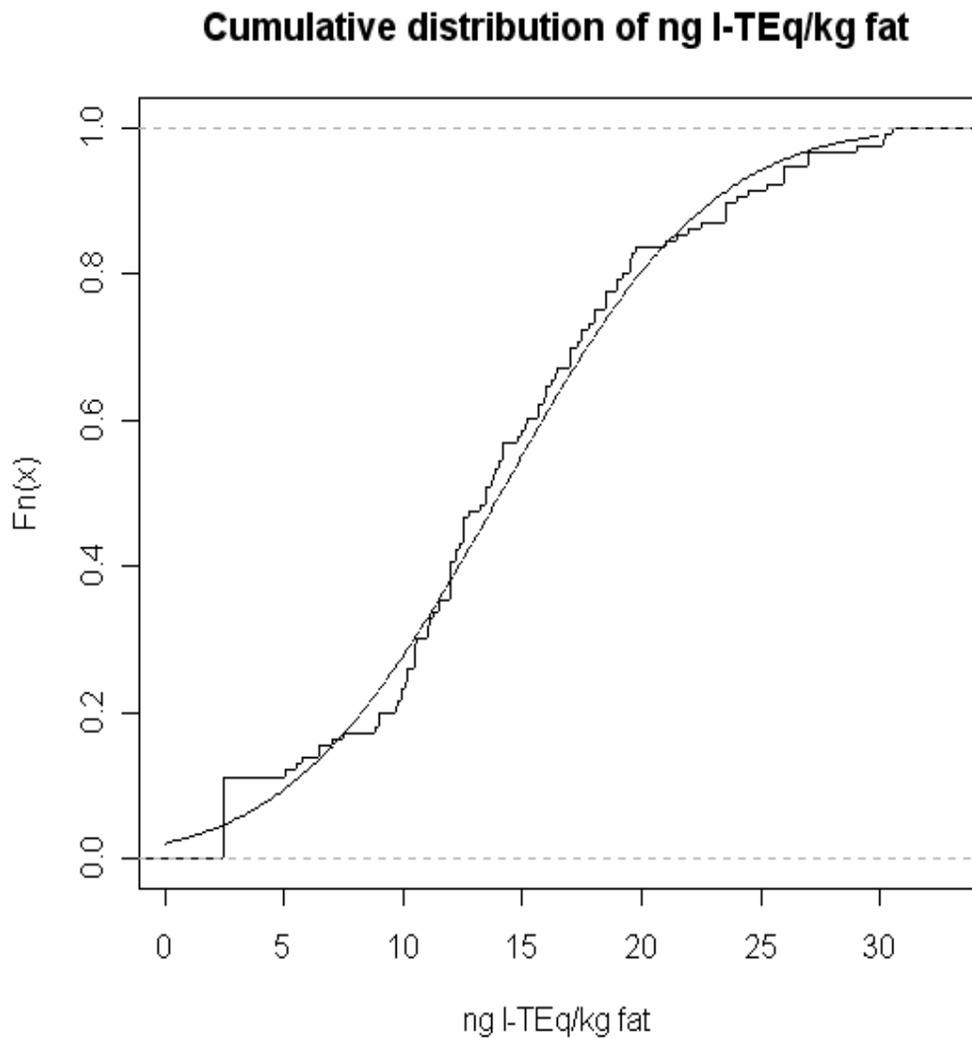
**Histogram of ng I-Teq/kg fat**



<sup>21</sup> Bundesumweltministerium (2002) Dioxine – Daten aus Deutschland, 3. Bericht: Daten zur Dioxinbelastung der Umwelt, Umweltbundesamt, Postfach 33 00 22, D-14191 Berlin, 123 S., ISBN 3-00-009326.

<sup>22</sup> Note that for illustration purposes, "I-Teq" (International Toxic Equivalence Factors) is used instead of the preferred WHO TEFs. However, the basic results and conclusions would be the same.

Figure 2



The histogram of Figure 1, when represented as cumulative data as shown above, is very close to the normal distribution depicted by the smooth curve. Statistical tests conclude that the parent distribution is normal with mean 14 and standard deviation 6.

We are now in a position to conduct statistical tests. We make simplifying assumptions: the normal distribution with standard deviation 6 holds at all time points of interest, only the mean may vary; and we are interested in only two time points, the base-line and first follow-up. The statistical testing scenario is given in Figures 3 and 4.

**Figure 3**

**STATISTICAL (CLASSICAL) PROBLEM**

- MEASUREMENTS AT TIME  $t_b < t_1 < t_2 < t_3 < \dots < t_n$
- HYPOTHESIS TO BE TESTED

$$H_0: \mu_{t_b} - \mu_{t_1} = 0$$

$$H_1: \mu_{t_b} - \mu_{t_1} \neq 0$$

We take measurements at various increasing time points denoted by the  $t$ 's above; as we simplify our analysis to two time points our hypothesis concerns only base-line and first follow-up. The Null Hypothesis is that of no change discernable from baseline to follow-up; the alternative hypothesis is that there is change (either positive or negative) between these two time points. It is important to recall that we test this hypothesis based upon a sample drawn from a large (if not infinite) population, so the results of our test are subject to random variation and probabilities. The possible outcome of our test and their probabilities are the subjects of Figure 4.

**Figure 4**

**STATISTICAL (CLASSICAL) PROBLEM**

	<i>STATE OF NATURE</i>	
	$\mu_{t_b} - \mu_{t_1} = 0$	$\mu_{t_b} - \mu_{t_1} \neq 0$
<b>H<sub>0</sub></b>	True  $1 - \alpha$	Error Type II  $\beta$
<b>H<sub>1</sub></b>	Error Type I  $\alpha$	True  $1 - \beta$

$1 - \beta$  Power of test

We are testing a statistical hypothesis. We may be either correct or incorrect in our conclusion; there are no guarantees. We base the conclusions on a sample drawn from a large population and, as such, are subject to random error. The above depicts the interaction between the (unknown but critical) State of Nature and the conclusions we draw about it are based upon our samples and statistical test. Four outcomes may be obtained based upon the assumptions made:

(a)  $1 - \alpha$ , referred to as the confidence level of the test, is the probability that the test concludes that the Null Hypothesis is true and it is, indeed, the State of Nature.

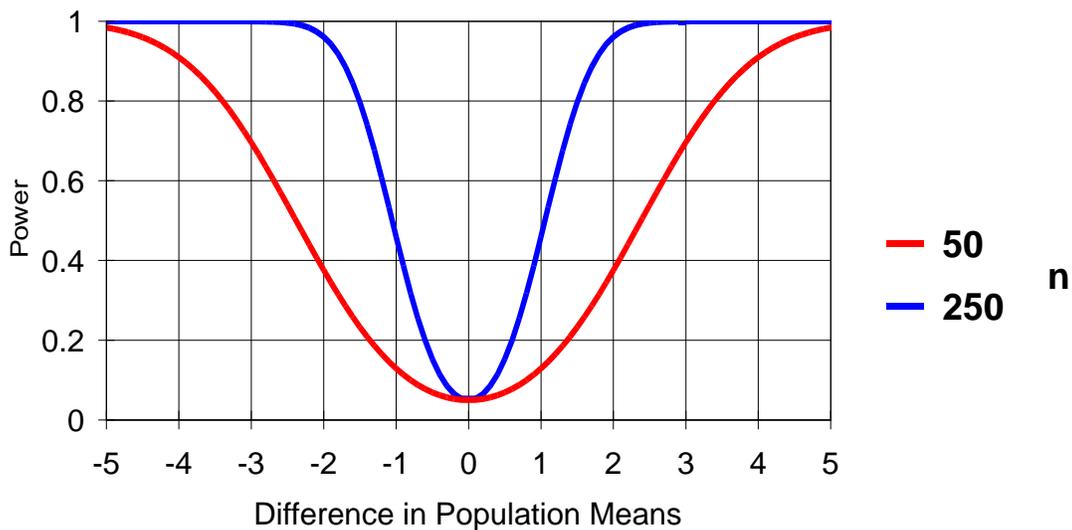
(b)  $\alpha$  referred to as the significance level, is the probability of a Type I error (rejecting a true Null Hypothesis). This test concludes that the Null Hypothesis is false and it is, indeed, not the State of Nature.

(c)  $\beta$  is referred to as the probability of a Type II error (accepting a false Null Hypothesis). The test concludes that the Null Hypothesis is true and it is, indeed, not the State of Nature.

(d)  $1 - \beta$ , referred to as the power of the test, is the probability that the test concludes that the Null Hypothesis is false and it is, indeed, the State of Nature

It is the power of the test that interests us most. We want to detect a significant difference via the statistical test when, in fact, it occurs in the State of Nature. The figures that follow illustrate power as a function of the other parameters in the testing scenario. This is intended to give an intuitive understanding of the interaction of all the relevant elements: magnitude of detectable difference, sample size, significance level, and variance of underlying samples.

**Figure 5**  
**Power as a function of sample size with  $\alpha = 0.05$**



In Figure 5 we see graphed as a function of detectable difference ‘Difference in Population Means’ and sample size of 50 or 250. Recall that the population mean of our underlying sample is 14. A 10% difference between base-line and first follow-up is represented by plus or minus 1.4.

A difference of 1.4 is detectable with probability 0.8 (quite high) with an underlying sample size of 250 but with probability 0.2 (quite low) with an underlying sample size of 50. If the detectable difference were increased to 20%, that is 2.8, we see that the probability of detection is about 0.7 (reasonably high) for a sample size of 50.

**Figure 6**  
**Power as a function of  $\alpha$  with sample size = 50**

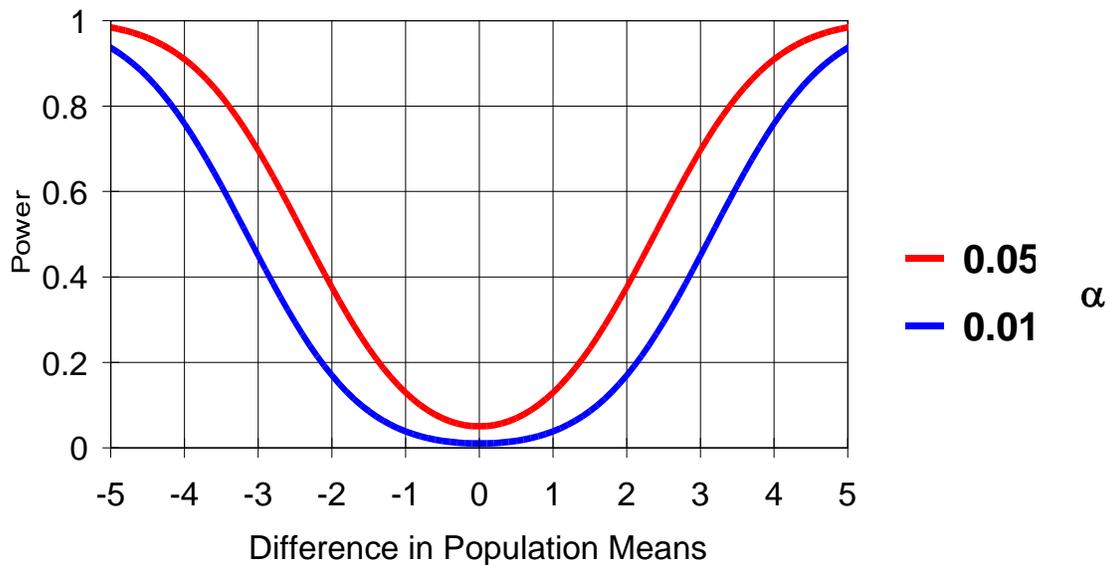
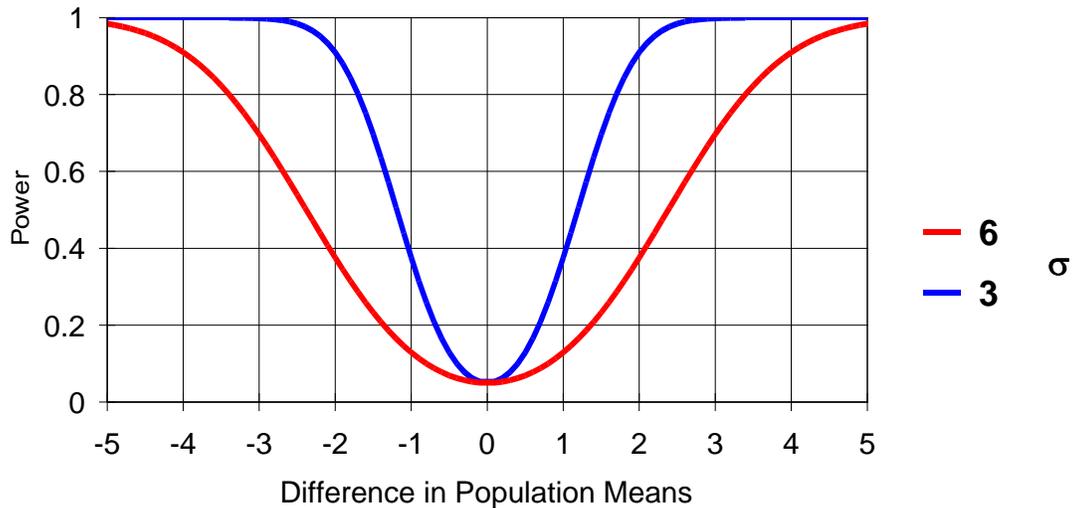


Figure 6 illustrates probability of detecting differences between base-line and follow-up as a function of  $\alpha$  the significance level or the probability of rejecting a true Null Hypothesis. Note that the  $\alpha$  level is the value over 0 difference in population means, the Null Hypothesis. Indeed, note that with a low  $\alpha$ , i.e. at 0.01 it is difficult to reject a Null Hypothesis, regardless of it being the State of Nature or not. The probability of rejecting the Null Hypothesis when the State of Nature exhibits a difference of 15% is only 0.2. The higher  $\alpha$  the more probable it is to reject the Null Hypothesis in favour of the alternative of detectable differences. Conversely, a higher  $\alpha$  denotes a higher probability of a Type I error.

**Figure 7**  
**Power as a function of standard deviation with  $\alpha = 0.05$  and sample size = 50**



Finally, in Figure 7 we examine the effect of a smaller standard deviation of the underlying sample on the ability to detect significant differences between base-line and follow-up measurements. This is relevant because we expect stratification by habitat, diet, age etc. to reduce the variability. If, in our test sample, the standard deviation were halved, from 6 to 3, and all else remains the same, sample size of 50 and  $\alpha$  level of 0.05, we see a great increase of power, the probability to detect differences when they are present in the State of Nature. The probability of detecting a difference of 10% is about 0.8 (quite high) when the standard deviation is reduced to 3; this is a great motivation to explore stratification.

### Conclusion

It is important to realize that the objectives of the survey and type of statistical analysis are determined before the sample is drawn. With this information one can study the interplay of the various elements, as above, to ensure that the sample should respond to the requirements. Although the simulation deals with individual measurements, the principles of sampling are generally the same for the pooled measurements.

It has been noted that we must foresee that the results of the survey should be subjected to scientific scrutiny. It may also be the fact that other investigators should attempt to replicate the results, within the statistical error limit. To allow replication, the sampling procedure must be thoroughly documented; the detail should serve as an audit trail of the selection of donors and their individual measurements. Finally, to allow for spatial comparisons, the general sampling procedures should be standardized from region to region. In this regard, documentation on the sampled population should be submitted to the ad hoc WHO Human Milk Survey Advisory Group (see Annex 8).

## QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS

### Fourth WHO-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		

<b>Section for National Coordinator</b>	
Individual Identification Code	Pool Identification Code
Based on established criteria, is the participant eligible?	
Yes <input type="checkbox"/> No <input type="checkbox"/>	
What is the status of donor in regard to the survey?	
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: Screening Questionnaire	
Name of Interviewer:	Date of interview (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 in your current area for 10 years?	
Yes <input type="checkbox"/>	No <input type="checkbox"/> *
If no, actual 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 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 :



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

<b>Section 4. To be completed by the Sample Collector</b>	
Name of 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 to sign the consent form? Yes <input type="checkbox"/> No <input type="checkbox"/> 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* <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? Male <input type="checkbox"/> Female <input type="checkbox"/>
4. Is your current weight different than your weight before pregnancy? Gained <input type="checkbox"/> Lost <input type="checkbox"/> Not changed <input type="checkbox"/>
5. Can you provide a sample now? Yes <input type="checkbox"/> Later <input type="checkbox"/> When? _____ At home <input type="checkbox"/> If you want to take the sample at home, do you have a refrigerator? Yes <input type="checkbox"/> No <input type="checkbox"/> ***

\* 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.

## SUMMARY INFORMATION ON THE WHO HUMAN MILK SURVEY<sup>23</sup>

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 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 this survey of POPs in human milk, the WHO through its GEMS/Food Programme will monitor all twelve POPs<sup>24</sup> currently covered by the Stockholm Convention to assist countries in their planning, management and evaluation of their POPs-reduction plans. This survey 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 survey will include samples from various regions of the world and will reflect different food consumption patterns. This survey will also support and, where feasible, strengthen national capabilities for the monitoring and sound management of POPs in food.

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.<sup>25</sup>

<sup>23</sup> 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.

<sup>24</sup> The twelve POPs presently included under the Convention are aldrin, DDT, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls, polychlorinated dibenzodioxins and polychlorinated dibenzofurans.

<sup>25</sup> WHO (2006) The International Code of Marketing of Breast-milk Substitutes. Frequently Asked Questions. Geneva, World Health Organization. ISBN 92 4 159429 2

## MODEL INFORMED CONSENT FORM

### Certificate of Consent

*I have been invited to take part in the research on WHO 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. The results of the surveys will help WHO and health officials in your country 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 or using a breast pump. We will provide training in how to express milk and how to use a breast pump. None of the questions that we will ask will be personal..

#### Compensation, provided to research subjects

As a form of appreciation for your time and input into the research, you will receive a small gift.

#### 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)

## 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 either by using manual expression or by using a human milk pump.

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. If using a pump, you should collect your milk in the container that comes with the pump (note that the pump will be delivered without the rubber teat).
- 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:

### Breast milk pump:

You should apply the pump to your breast and continue to pump until the milk flow declines to a drip. You may wish to use the pump at the same time your infant is nursing on the other breast as this helps release your milk.

### 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 health centre as soon as possible and protected from high temperatures during the transport. If refrigeration is not available in your home, your collection jar will 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.

<b>Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants</b> <b>SUMMARY INFORMATION FOR A POOLED SAMPLE</b> <b>(Based on confidential questionnaires from mothers donating human milk samples)</b>		
Country	Pool Identification code	Number of mothers in the pool
1. Ages of the mothers Mean <input style="width: 100%; height: 20px;" type="text"/> Range <input style="width: 100%; height: 20px;" type="text"/>		2. Mother's height (in cm) Mean <input style="width: 100%; height: 20px;" type="text"/> Range <input style="width: 100%; height: 20px;" type="text"/>
3. Mother's weight before pregnancy Mean (in kg) <input style="width: 100%; height: 20px;" type="text"/> Range (in kg) <input style="width: 100%; height: 20px;" type="text"/>		4. Child's age in weeks at sampling Mean <input style="width: 100%; height: 20px;" type="text"/> Range <input style="width: 100%; height: 20px;" type="text"/>
5. Area of residence during last 10 years: (% of the total mothers of the pool) Urban <input style="width: 50px; height: 20px;" type="text"/> rural <input style="width: 50px; height: 20px;" type="text"/>		
6. Mother's <b>dietary habits</b> (% of total mothers in the pool) Mixed diet <input style="width: 50px; height: 20px;" type="text"/> Vegetarian but with milk and egg <input style="width: 50px; height: 20px;" type="text"/> Strictly vegetarian <input style="width: 50px; height: 20px;" type="text"/> Other <input style="width: 50px; height: 20px;" type="text"/>		
7. Mother born in the country (% of total mothers in the pool) <input style="width: 100%; height: 20px;" type="text"/>		8. Mother raised by breastfeeding (% of total mothers in the pool) <input style="width: 100%; height: 20px;" type="text"/>

<p>9. Mother's mother born in the country (% of total mothers in the pool)</p> <div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 10px;"></div>	<p>10. Mothers working before pregnancy (% of total mothers in the pool)</p> <div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 10px;"></div>																																																	
<p>11. Exposure to DDT from inside house spraying in order to prevent mosquitoes (% of total mothers in the pool)</p> <div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 10px;"></div>	<p>12. Mothers whose current weight that is less than their weight before pregnancy (% of total mothers in the pool)</p> <div style="border: 1px solid black; height: 30px; width: 100%; margin-top: 10px;"></div>																																																	
<p>13. Mother's consumption of food (% of mother in the pool)</p> <table border="1" style="width: 100%; border-collapse: collapse; text-align: center;"> <thead> <tr> <th style="width: 15%;"></th> <th style="width: 15%;">Fish</th> <th style="width: 15%;">Marine Mammals</th> <th style="width: 15%;">Seafood other than fish and mammals</th> <th style="width: 15%;">Milk and milk products</th> <th style="width: 15%;">Meat and poultry</th> <th style="width: 15%;">Eggs</th> </tr> </thead> <tbody> <tr> <td style="text-align: left;">Never</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: left;">Less than once a week</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: left;">Once a week</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: left;">Twice or less a week</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: left;">More than twice a week but not every day</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: left;">Every day</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table>			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						
	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																																																		
<p>14. Type of fish mother consumed most often (% of the mother in the pool)</p> <table style="width: 100%; margin-top: 10px;"> <tr> <td style="text-align: center; width: 33%;">Fish from the sea</td> <td style="text-align: center; width: 33%;">Fresh fish</td> <td style="text-align: center; width: 33%;">Both</td> </tr> <tr> <td style="text-align: center;"><div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div></td> <td style="text-align: center;"><div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div></td> <td style="text-align: center;"><div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div></td> </tr> </table>		Fish from the sea	Fresh fish	Both	<div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div>	<div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div>	<div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div>																																											
Fish from the sea	Fresh fish	Both																																																
<div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div>	<div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div>	<div style="border: 1px solid black; width: 50px; height: 25px; margin: 0 auto;"></div>																																																
<p>15. POPs analyses requested besides the twelve (12) Stockholm POPs:</p> <p>None _____ List _____</p>																																																		
<p>Date (dd/mm/yyyy)</p>	<p>Name of National Coordinator</p>	<p>Signature</p>																																																

## ESTIMATED TIMELINE AND BUDGET

In conducting the first sampling of the proposed survey, each participating country should anticipate following a similar schedule with similar expenses as estimated below. In subsequent sampling periods, only marginal savings can be expected. It must be emphasized for all parties and involved partners that it is not possible to save time at later steps when previous steps (such as selection of National Coordinator, clarification of budget questions, collection of samples, etc.) are delayed. The shorter these steps, the higher the probability that results can be obtained on schedule.

### Timeline (based on 50 individual samples and 1 pooled sample)

#### **Month 1-6: Basic organization**

After announcement of survey, selection of National Coordinator, final clarification of availability of budget, candidate laboratory participation in the proficiency test organized by the WHO Reference Laboratory and selection of national or regional laboratory for analysis of individual samples.

#### **Month 7 - 10: Pre-selection of individual samples\***

Month 7-8: interviewing of up to 250 possible participants

- Planning and coordinating by National Coordinator: 5 months x 20 days x 8 hr = 800 hrs
- Training of interviewers on prenatal information on breastfeeding and pumping and/or hand expression skills = 10 hrs or more
- Interviewing: 0.5 hr x 250 questionnaires = 125 hrs

Month 9-10: Selection of 50 qualified participants as well as about 10 alternates

Reviewing, selecting and notifying participants on the basis of the questionnaire 0.25 hr x 250 questionnaires = 65 hrs

#### **Months 11 - 17: Sampling of pre-selected individuals and sample handling**

Month 11 -17: Collection and handling of samples

- Interviewing and collecting samples 1 hr x 50 samples = 50 hrs
- Preparing of individual and pooled samples = 16 hrs
- Preparing for shipping = 4 hrs

**Months 7 - 12: Preparation of proficiency test samples by the WHO Reference Laboratory for laboratories and shipment to laboratories selected by the National Coordinator for analysis of the individual samples for *analytically simple POPs*.**

**Month 18-24: Sample analysis (see below for cost estimates)**

**Month 25 - 26: Evaluation and report preparation = 40 hrs**

---

\* Alternatively, direct collection of individual samples at postnatal clinics can advance the schedule by 4 months.

## Table of Estimated Human Resources and Costs

<b>Item</b>	<b>Estimated Human Resources and Costs</b>	<b>Justification</b>
Personnel	Planning and coordination: 800 hrs Breastfeeding counselling training: 40 hrs Collection and handling of samples: 70 hrs  Total : 910 hrs  Prenatal selection option: <ul style="list-style-type: none"> <li>• Interviewing: 125 hrs</li> <li>• Selection of participants: 65 hrs</li> </ul>	Must provide sufficient skills for supporting breastfeeding women
Documentation expenses	Translation, printing and distribution	National protocol, questionnaire, informed consent, summary information, etc.
Travel	\$ 1000	Regional survey protocol preparation and review meeting and local travel to clinics
Supplies/equipment	Collection jars 50 x \$15 = \$750 Container for pooled sample \$80 Breast pumps (optional) 50 x \$10 = \$500 Shipping: country-specific costs \$1000	Chemicals and glassware have been included for the collection and extraction of individual milk samples
Incentives	T-Shirt 50 x \$10 = \$ 500	In order to facilitate the survey
Other	\$ 1000	Costs associated with publishing the results and administrative fee
Analysis	Analysis of 1 pooled sample ( <i>analytically simple and complex POPs</i> ): \$ 2000 Analysis of 1 pooled sample for optional POPs: <ul style="list-style-type: none"> <li>- PBDEs: \$ 300</li> <li>- Polybrominated and mixed halogenated dioxins and furans: \$ 500</li> <li>- HBCDs: \$ 500</li> </ul> Analysis of 50 individual samples ( <i>analytically simple POPs only</i> ): \$18 000 (if performed in Europe)	

**ADDITIONAL REFERENCES AND READING**

WHO (2002) Safety evaluation of certain food additives and contaminants (2002) Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls. Food Additives Series: 48, World Health Organization, Geneva

WHO (2002) Global assessment of the state-of-the-science of endocrine disruptors, WHO/ILO/UNEP International Programme on Chemical Safety, World Health Organization, Geneva

LaKind, J and Berlin, CM Jr. (2002) Technical workshop on human milk surveillance and research on environmental chemicals in the United States: An overview. *J. Toxicol. Environ. Health A.* 65:1829 - 183 (This was a special issue with several useful articles related to human milk monitoring)

LaKind, J, Berlin, CM Jr and Bates, MN (2005) Overview: Technical workshop on human milk surveillance and biomonitoring for environmental chemicals in the United States, *J. Toxicol. Environ. Health A.* 68:1683 - 1689. This was a special issue with several useful articles related to human milk monitoring, including:

Fenton S et al. Collection and use of exposure data from milk biomonitoring in the United States.

LaKind J et al. Human milk biomonitoring data: Interpretation and risk assessment issues.

Wang R et al. Human milk research for answering questions about human health.

Berlin CM Jr et al. Methodologic considerations for improving and facilitating human milk research.

Berlin CM Jr et al. Conclusions and recommendations of the expert panel

Van Leeuwen FXR and Malisch R (2002) Results of the third round of the WHO-coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. *Organohalogen Compounds*, 56: 311-316.

Malisch R. and Moy G (2006) Fourth round of WHO-coordinated exposure studies on levels of persistent organic pollutants in human milk. *Organohalogen Compounds*. (Vol 68 - in press)

Norén K and Meironyte D (2002) Certain organochlorine and organobromine contaminants on Swedish human milk in perspective of past 20-30 years. *Chemosphere* 2002; 40 (9-11): 1111-1123.

Pronczuk J, Moy G and Vallenias C (2004) Breast Milk: An Optimal Food. *Environmental Health Perspectives*, 112 (13): A722-723. Available online: <http://ehp.niehs.nih.gov/docs/2004/112-13/EHP112pa722PDF.PDF>

## WHO TEF

Compound	WHO 1998 TEF	WHO 2005 TEF*
<b>Polychlorinated dibenzodioxins</b>		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	<b>0.0003</b>
<b>Polychlorinated dibenzofurans</b>		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	<b>0.03</b>
2,3,4,7,8-PeCDF	0.5	<b>0.3</b>
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	<b>0.0003</b>
<b>Dioxin-like polychlorinated biphenyls</b>		
<b><u>Mono-ortho PCBs</u></b>		
IUPAC No. 105	0.0001	<b>0.00003</b>
IUPAC No. 114	0.0005	<b>0.00003</b>
IUPAC No. 118	0.0001	<b>0.00003</b>
IUPAC No. 123	0.0001	<b>0.00003</b>
IUPAC No. 156	0.0005	<b>0.00003</b>
IUPAC No. 157	0.0005	<b>0.00003</b>
IUPAC No. 167	0.00001	<b>0.00003</b>
IUPAC No. 189	0.0001	<b>0.00003</b>
<b><u>Non-ortho PCBs</u></b>		
IUPAC No. 77	0.0001	0.0001
IUPAC No. 81	0.0001	<b>0.0003</b>
IUPAC No. 126	0.1	0.1
IUPAC No. 169	0.01	<b>0.03</b>

\* Numbers in bold indicate a change in TEF value

Reference - *Van den Berg et al:*

The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds