Survey of Human Milk for Persistent Organic Pollutants in Cooperation with WHO

National Protocol

for the Republic of Korea

National Institute of Toxicological Research

Seoul, Korea

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Contents

1. Background

Persistent organic pollutants (POPs) are a group of chemicals that have been intentionally or inadvertently produced and introduced into the environment. Due to their stability and transport properties, POPs 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 are found 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.

Since 1976 the World Health Organization through its GEMS/Food Programme has collected and evaluated the levels of POPs in foods, including human milk. Among the relevant matrices for assessment of body burdens for POPs, human milk is recognized as the preferred matrix because it has several important advantages. Biomonitoring of human milk data can provide information on the exposure of the mother as well as the infants. In addition, such information provides guidance in the need for measures to reduce levels of these substances in food.

With the ratification of the Stockholm Convention on POPs in early 2004, the international community including South Korea signaled its commitment to reduce or eliminate production and emission of twelve important POPs[1] into the environment and ultimately, the human body. In May 2005, WHO and the United Nations Environment Programme (UNEP) entered into a Memorandum of Agreement for the coordination of human milk survey for the purpose of the Stockholm Convention.

To ensure reliability and improve comparability, WHO has routinely carried out inter-laboratory analytical quality assurance studies of POPs. 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.[2]

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[1] 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.

[2] Inter-laboratory quality assessment of levels of PCDDs, PCDFs and dioxin-like PCBs in human milk and blood plasma, WHO

WHO encourages exclusive breastfeeding for six months for the vast majority of infants, followed by continued breastfeeding with appropriate complementary foods for up to two years or beyond.[3] Evidence for the health advantage of breastfeeding and scientific evidence to support breastfeeding has continued to increase.

This document is developed as a National Protocol for the monitoring of POPs in human milk in Korea based on the *Guidelines for Developing a National Protocol* developed according to the advice and suggestions of the ad hoc WHO Human Milk Survey Advisory Group. This protocol would serve the needs of the Stockholm Convention. Also, human milk survey in Korea supports the notion of WHO encouraging breastfeeding. 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 in Korea.

2. Aims of the protocol

The main aims of the 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 food

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Report EUR/00/5020352, WHO Regional Office for Europe, Copenhagen, 2000

[3] WHO (2006) The International Code of Marketing of Breast-milk Substitutes. Frequently Asked Questions. Geneva, World Health Organization. ISBN 92 4 159429 2

supply. Also, these surveys are intended to examine levels within countries over time.

3. General principles

This protocol is prepared based on the following general principles.

- Breastfeeding should be protected, promoted and supported.
  Annex 1 provides a perspective on why breastfeeding is important, which should be required reading by all persons administering and participating in the survey.
- 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.

This national protocol adheres as closely to WHO protocol as possible with the least modification due to the characteristics of Korean population and the feasibility of the subsequent survey and meets the aims of the survey as mentioned above. This protocol also meets ethical requirements for human subjects, including informed consent of donors and confidentiality in Korea.

4. Contents of a national protocol

4.1 Pooled versus individual samples

Analytically simple POPs (Annex 2)

Individual samples should be analyzed for the basic pesticide POPs and marker PCBs by a method that uses gas chromatography/electron capture detector. The analyses of individual samples can provide information on the distribution of exposures and on factors possibly contributing to exposure. Also, such data are essential to statistically validate changes in levels of POPs over time. As an internal quality control check, pooled sample will be analyzed for *analytically simple POPs* because the average value from individual samples should be equal to the pooled sample value.

Analytically complex POPs (Annex 2)

Pooled samples will be used to monitor the levels of PCDDs, PCDFs and dioxin-like PCBs in human milk. For complex POPs analyses, a pooled sample from subjects in Korea will be sent to the WHO

reference laboratory for POPs in human milk.

Other POPs (Annex 2)

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 resources available. However, other POPs in this criterion are not analyzed in individual samples in Korea but requested to analyze in a pooled sample in WHO reference laboratory.

4.2 Number of samples

As of July 2007, Korean population was recorded to over 48 million according to Korea National Statistical Office; therefore, minimum of 50 individual samples will be initially collected from three major cities (Seoul, Kwangju and Busan) in Korea. Considering the statistical aspects of the survey, the numbers of sample may be increased.

4.3 Criteria and selection of participants

4.3.1 Selecting procedure

For participant recruitment, posters are distributed at health clinics providing prenatal and postnatal services and in residential areas for the designated cities. Collection of samples is conducted at the health clinics or mothers' home. Mother is generally explained the background and the purpose of the survey as described in the summary information (see Annex 4) and completes a questionnaire Sections 1-2-3-4 (see Annex 3). National Coordinator reviews the questionnaires of the potential donors and selects 50 potential donors that best meet the criteria for inclusion in the survey. Informed consent form should be completed at the time of sampling.

4.3.2 Criteria for selecting participants

A general starting point will include the following;

- Mother should be primiparae.
- Mother should be between 25 and 35 years of age[4].
- 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.

[4] 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.

- 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.

4.4 Collection of samples

4.4.1 Collection of milk

Sampling can be carried out between 3 to 8 weeks (21 days to 2 months) after delivery. At least 50 ml of milk in total should be collected by hand expression[5] after a feeding or while infant is nursing on the other breast. The sample should be collected directly to the collecting jar with a protected screw cap to collect. Under the supervised condition, mother provides the sample when the interviewer/sample collector visits at her home or clinic.

If it is her wish, the mother may collect the sample at home later. If so, she should be given detailed instructions for taking, storing and transporting of milk samples (see Annex 6). Sample collection jars should be labeled with the donor's individual identification code and not the name of the mother. Milk samples may be stored in the refrigerator at about 4 °C for a maximum of 72 hours, or for longer times in the freezer at - 20 °C.

4.4.2 Collection of questionnaire

At the time of collecting milk, interviewer visits at the clinics or mother's home and collect the questionnaire. In principle, interviewer will ask mother to fill out the questionnaire and to sign the informed consent form. In case mothers are not able to fill out the questionnaire by themselves, the interviewer may read the questions and check the answers for themselves. Note that interviewer should not arbitrarily interpret and explain the questions to the mothers.

4.5 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. Pregnant women are screened for a number of

[5] 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

infectious diseases so that their health status can be evaluated. In case of possible HIV-positive donors, they would be excluded from this survey since the potential weight loss of the donors could be an issue as well as the biosafety of the samples.

4.6 Transporting of samples

Each individual and pooled sample should be labeled with a unique identification code. The 50 samples containing 50 ml each should be frozen at -20°C, packaged in dry ice and sent to the laboratory designated by the National Coordinator. 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. Receipt of the package in the laboratory should be confirmed.

4.7 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 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 in the national laboratory in Korea, and the remaining pooled sample of 450 ml will be analyzed for *analytically complex POPs* by WHO Reference Laboratory. Fifteen ml of the remained sample could be used for the additional individual analyses of simple POPs in Korea and the remainders should be pooled that will be sent to WHO for its Global Human Milk Bank.

4.8 Analysis of individual samples and capacity building

4.8.1. Analytical procedures

Using 25 ml of human milk, the 50 individual samples should be analyzed for pesticide POPs and marker PCBs at the national laboratory in Korea. A number of analytical methods using gas chromatography with electron capture detector (ECD) 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 analyzed and results reported on a fat basis.

4.8.2. Quality controls for analysis

For the proficiency test, 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. 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 analyzing the individual samples. In addition, the laboratory should analyze an aliquot of the pooled sample for analytically simple POPs in order to compare results with the WHO Reference Laboratory. Note that mean results of individual analyses can be compared with the result of the pooled sample analyzed by the WHO Reference Laboratory. All analytical results will be reported on a fat basis.

4.9 Analysis of pooled samples

The 450 ml pooled sample will be analyzed for *analytically simple and complex POPs*, including PCDDs, PCDFs and dioxin like PCBs. A complete list of *analytically simple and complex POPs* as well as some optional POPs is given in Annex 2.

The 450 ml of pooled sample will be analyzed 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

4.10 Data handling and assessment considerations

For results below the limit of determination (LOD), one-half the LOD 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[6].

All results are reported in a format that is compatible with the GEMS/Food data structure for individual contaminants in food (see http://www.who.int/foodsafety/publications/ chem/gems_instructions /en/ index.html).

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)[7]. Dissemination of results in

[6] 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/

[7] The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and

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.

5. Ethics

Based on national requirements, a National Coordinator should decide whether to provide donors with the results of individual and/or pooled samples. 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.

6. Financial aspects

NITR 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. NITR will also be responsible for the preparation, and analysis of pooled samples. NITR should provide adequate facilities and other in-kind contributions to facilitate the collection, preparation and handling of the samples. WHO will also support the cost of the proficiency testing scheme for pesticide POPs and marker PCBs.

Dioxin-like Compounds, *Martin van den Berg,et al.,* Tox Sci Advance, 2006. See 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.

ANNEX 1

THE VALUE OF BREASTFEEDING [8]

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 higher than 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 higher than 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, diarrhea 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.

[8] The National Coordinator may simplify this information depending on the needs and educational status of women involved in the survey.

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 amenorrhea [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.

ANNEX 2

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-heptabromdiphenyl 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)

ANNEX 3

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 e-mail	Today's Date (dd/mm/yyyy)
Address		
Status of donor in regard to the survey (completed by National Coordinator) Selected □ Reserve □		
Individual Identification Code		

Section 2: **Screening Questionnaire:To be completed by the interviewer/sample collector**	
Name of Interviewer/collector:	Date of interview/collection (dd/mm/yyyy):
Place of interview/collection: (dd/mm/yyyy):	Clinic of Collection:
1. Are you breastfeeding your child? Yes □ No □	
2. Is this your first child? Yes □ No □	
3. Is your child born as a single child? (not twins) Yes □ No □	
4. Did you have a normal healthy pregnancy? Yes □ No □	
5. Have you lived in your current area for 10 years? Yes □ No □ * If no, actual number of years _____	
6. Are you between 25 and 35 years old? Yes □ No □ * If no, date of birth _____(dd/mm/yyyy)	

7. Do you live near incinerators, pulp and paper industries, metal industries or where chemicals are produced Yes □ No □

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

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

Section 3: Health History Questionnaire	
Date of Birth (dd/mm/yyyy)	Age(yrs)
Height (cm)	Weight before pregnancy (kg)
1. What was your delivery date (dd/mm/yyyy)?	
2. What was the type of your delivery? Natural birth □ Caesarean □	
3. Where have you been residing during last 10 years: urban (city) □ rural (countryside) □ Describe the actual periods of residence on the area _____years	
4. How would you describe your dietary habits before pregnancy? Mixed diet □ Vegetarian but with milk and eggs □ Strictly vegetarian □ Other □	

	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						

5. How often, on average, did you eat following foods before pregnancy?

5.1 What types of fish do you consume most often?

Fish from the sea □ Freshwater fish □ Both □

Please state the species if known :

5.2 Were your dietary habits changed after pregnancy?

Yes □ No □

If yes, describe major changes briefly:_____

6. Were you born in Korea?

Yes □ No □

7. Was your mother born in Korea?

Yes □ No □

8. Were you breastfed?

Yes □ No □ Do not know □

If you know, for how long? _____

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

Yes □ No □

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

10. Has the inside of your house been sprayed with DDT in order to prevent mosquitoes? Yes □ No □ Do not know □ If yes, when? _____
11. Do you smoke? Yes □ No □
11.1 If yes, how many cigarettes do you smoke per day? _____
11.2 If no, have you ever smoked? Yes □, please state the quitting periods:_____ years No □

Section 4. Postnatal Information
1. Are you prepared to sign the consent form? Yes □ No □ If yes, attach signed consent form. If no, mother is not eligible to participate in survey.
2. How old is your infant? less than 3 weeks* □ 3-4 weeks □ 5-8 weeks □ more than 8 weeks □**
3. What is the gender of your infant? Male □ Female □
4. What was the birth weight of your baby? _____ kg
5. What is the weight of your baby? _____ kg
6. Did you feed colostrums to your baby? Yes □ No □
7. Are you taking any medicine? Yes □ No □ If yes, please describe the medicines: _____
4. Is your current weight different than your weight before pregnancy? Gained □ Lost □ Not changed □
5. Can you provide a sample now? Yes □ Later □ When? _____ At home □

* 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.

ANNEX 4

SUMMARY INFORMATION ON THE WHO HUMAN MILK SURVEY[9]

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

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

Meeting under the auspices of the United Nations Environment Programme (UNEP), parties to the Convention have identified human milk as one of the core matrices to be monitored to evaluate the impact of the Stockholm Convention in reducing emissions of POPs. In conducting this survey of POPs in human milk, the WHO through its GEMS/Food Programme will monitor all twelve POPs[10] 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

[9] 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.

[10] 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.

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 analyzed 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 tends as countries implement measures to reduce the emission of POPs into the environment.

At the same time, evidence for the health advantages of breastfeeding has continued to increase. On a population basis, exclusive breastfeeding for six months is the recommended feeding mode for the vast majority of infants, followed by continued breastfeeding with appropriate complementary foods for up to two years or beyond[11].

[11] WHO (2006) The International Code of Marketing of Breast-milk Substitutes. Frequently Asked Questions. Geneva, World Health Organization. ISBN 92 4 159429 2

ANNEX 5

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 Program 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 manual expression. The sample will be collected at the most convenient health clinic or at your home. Your sample will be analyzed 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. We will provide training in how to express milk. 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 Dr. Yoon Hae-Seong (National Coordinator in Korea). 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:

Yoon, Hae-Seong, Ph.D
Human exposure assessment division
National Institute of Toxicological Research
Korea Food and Drug Administration
Tel) 82-2-380-1825
E-mail) hsyoon0956@kfda.go.kr

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to

withdraw from the study until my sample has been pooled with others. If I choose to withdraw from the study, I understand that I can do so without in any way affecting my medical care. I also consent that any excess sample of breast milk may be kept for related surveys in the future.

Print Name of Participating Mother **Date and Signature of Participating Mother**

_____ **_____**

___/___/___ (*dd/mm/yy*)

If illiterate

Print Name of Independent Literate Witness Date and Signature of Witness

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

_____ **_____**

___/___/___ (*dd/mm/yy*)

Print Name of Researcher **Date and Signature of Researcher**

_____ **_____**

___/___/___ (*dd/mm/yy*)

ANNEX 6

GUIDANCE FOR MOTHERS COLLECTING MILK SAMPLES AT HOME

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

How to collect samples:

You may collect the sample by using manual expression.

You have already been given instructions on these methods, but remember:

• You should not use any other vessel for collecting milk. You must not use cups or other bottles you may have at home. You should collect your milk directly into the small jar provided to you.

• You should keep your breasts and hands as clean as possible, but soap should be avoided because they may contain chemicals that interfere with the analysis. When it is necessary to use soap, you should rinse your breasts and hands thoroughly with clean water.

• You should avoid using ointments on your nipples before collecting your milk. If you have used ointment that day, you should wash your nipples with soap and thoroughly rinse with clean water.

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

Manual method:

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

When to collect your sample:

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

Storage and transport of your sample:

If you do not collect 50 ml at once, the partial sample may be stored in the refrigerator and sampling can be continued the next day. If 50 ml is still not collected, the sampling may be continued for a third day. However, after 3 days sampling should be stopped and the sample frozen if possible. The sample should

be delivered to the 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 to the clinic the next day. You should be careful to keep the jar containing the chemical out of the reach of children as it is dangerous if eaten.

ANNEX 7

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

SUMMARY INFORMATION FOR A POOLED SAMPLE

(Based on confidential questionnaires from mothers donating human milk samples)

Country	Pool Identification code	Number of mothers in the pool

1.Ages of the mothers Mean Range	2.Mother's height (in cm) Mean Range
3.Mother's weight before pregnancy Mean (in kg) Range (in kg)	4. Child's age in weeks at sampling Mean Range
5. Area of residence during last 10 years: (% of the total mothers of the pool) Urban □ rural □	
6. Mother's dietary habits (% of total mothers in the pool) Mixed diet Vegetarian but with milk and egg Strictly vegetarian Other	
7. Mother born in Korea (% of total mothers in the pool)	8. Mother raised by breastfeeding (% of total mothers in the pool)
9. Mother's mother born in Korea (% of total mothers in the pool)	10. Mothers working before pregnancy (% of total mothers in the pool)
11. Exposure to DDT from inside house spraying in order to prevent mosquitoes (% of total mothers in the pool)	12. Mothers whose current weight that is less than their weight before pregnancy (% of total mothers in the pool)
13. Mother's consumption of food (% of mother in the pool)	

	Fish	Marine mammals	Seafood other than fish and marine	Milk and milk products	Meat and poultry and derived products	Eggs
Never						
Less than once a week						
Once a week						
Twice a week						
More than twice a week but not every day						
Every day						

14. Type of fish mother consumed most often (% of the mother in the pool)

Fish from the sea ☐ Fresh fish ☐ Both ☐

15. POPs analyses requested besides the twelve (12) Stockholm POPs:

None _____ List _____

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

ANNEX 8

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 milkresearch.
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 Vallenas 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