

Trinational Biomonitoring Study



Assessment of Persistent Organic Pollutants and Selected Metals in the Blood of First-Birth Mothers in Southern Canada and Mexico and in Women of Reproductive Age in the United States

This study was prepared by the Secretariat of the Commission for Environmental Cooperation. See Acknowledgments for detailed information about contributions to this study. The information contained herein does not necessarily reflect the views of the CEC, or the governments of Canada, Mexico or the United States of America.

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Commission for Environmental Cooperation, 2011



Publication Details

Publication type: *Report*
Publication date: *October 2011*
Original language: *English*
QA08.34

Please cite as :

CEC. 2011. *Trinational Biomonitoring Study: Assessment of Persistent Organic Pollutants and Selected Metals in the Blood of First-Birth Mothers in Southern Canada and Mexico and in Women of Reproductive Age in the United States*. Montreal: Commission for Environmental Cooperation.

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Acknowledgements

The Commission for Environmental Cooperation wishes to recognize the dedicated experts responsible for developing this Trinational Blood Biomonitoring study: Assessment of Persistent Organic Pollutants and Selected Metals in the Blood of First Birth Mothers in Southern Canada and Mexico and in Women of Reproductive Age in the United States:

- Mauricio Hernandez, *Instituto Nacional de Salud Pública, Secretaría de Salud*
- Leonora Marro, Health Canada
- Larry Needham, co-author, Centers for Disease Control and Prevention
- Horacio Riojas, co-author, *Instituto Nacional de Salud Pública*
- Sandra Rodriguez, *Instituto Nacional de Salud Pública*
- Jay Van Oostdam, co-author, Health Canada
- Jean-Philippe Weber, co-author, consultant
- Lee-Yang Wong, Centers for Disease Control and Prevention

The Commission for Environmental Cooperation also wishes to recognize the valuable contributions of the following individuals and institutions in the development of this study:

- The heads and staff of: *Hospital de Especialidades del niño y la mujer Querétaro, Hospital General de Cuautitlán, Centro de Salud de la Cabecera Municipal de Tultitlán Centro, Hospital Materno Infantil Mérida, Hospital General Coatzacoalcos, Hospital Universitario Monterrey, Hospital Integral Hermosillo, Hospital Zona “Yanga,” Hospital General Ciudad Obregón, and Hospital Civil Guadalajara.*
- The lead researchers (Drs W. Foster, A. Chung, G. Graves, J. Jarrell, M. Walker, and K. Davis) and their staff at the following medical centers, respectively: McMaster University, University of British Columbia, Dalhousie

University, University of Calgary, University of Ottawa, and Health Canada.

- The contributing mothers without whom this study could not have taken place.

This study was made possible through the support of officials from Environment Canada, Health Canada, *Instituto Nacional de Salud Pública*, *Secretaría de Salud*, *Secretaría de Medio Ambiente y Recursos Naturales*, *Instituto Nacional de Ecología – Centro Nacional de Investigación y Capacitación Ambiental*, the Centers for Disease Control and Prevention, the United States Environmental Protection Agency and the World Bank.

This report was prepared with the aid of the CEC Secretariat, including Luke Trip, Program Manager for Sound Management of Chemicals, Lucie Robidoux, Coordinator of the Environmental Monitoring and Assessment project of the Sound Management of Chemicals program, and Erika Hercules, program assistant. The publications staff, of Douglas Kirk, Jacqueline Fortson, and Johanne David, under the guidance of Jeffrey Stoub, carried out the demanding job of editing, translating and publishing the report, including a summary in three languages.

It is with great sadness that the above contributors to this study wish to dedicate it to the memory and devotion of Dr. Larry Needham, co-author and principal supporter of this work, who died in October 2010 after having seen it through its various stages. Larry's strong belief in the value of the study enabled him to surmount his personal tribulations and continue working on it until the last few weeks before his death.

Acronyms

CCAYAC	<i>Comisión de Control Analítico y Ampliación de Cobertura</i> (Commission for Analytical Control and Extension of Coverage)
CDC	Centers for Disease Control and Prevention
Cenica	<i>Centro Nacional de Investigación y Capacitación Ambiental</i> (National Center for Environmental Research and Training)
CHMS	Canadian Health Measures Survey
Cofepris	<i>Comisión Federal para la Protección contra Riesgos Sanitarios</i> (Federal Commission for Risk Protection)
EPW	expectant primiparous women (women in their first pregnancy)
EQA	external quality assurance
EQAS	external quality assessment scheme
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane [either alpha-, beta-, or gamma-(lindane) forms]
INE	<i>Instituto Nacional de Ecología</i> (National Institute of Ecology)
INSP	<i>Instituto Nacional de Salud Pública</i> (National Institute of Public Health)
INSPQ	<i>Institut national de santé publique du Québec</i> (National Institute of Health of Quebec)
LOD	limit of detection
NHANES	US National Health and Nutrition Examination Survey
OCP	organochlorine pesticides
PAHO	Pan American Health Organization
PBT	persistent, bioaccumulative, toxic chemicals
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzo- <i>p</i> -dioxins
PCDF	polychlorinated dibenzofurans
POPs	persistent organic pollutants
p,p'-DDE	p,p'-dichlorodiphenyl-dichloroethylene
Proname	<i>Programa Nacional de Monitoreo y Evaluación de Sustancias Tóxicas, Persistentes y Bioacumulables</i> (National Monitoring and Environmental Evaluation Program)
SMOC	Sound Management of Chemicals program
TEF	toxic equivalency factor
TEQ	toxic equivalent
UASLP	<i>Universidad Autónoma de San Luis Potosí</i>

Executive Summary

Human exposure to persistent organic pollutants (POPs) and certain toxic metals in our environment is a matter of serious concern because of their potential for causing deleterious effects on our health. An assessment of the degree of pollutant exposure in different sections of the three North American countries is thus of great importance. As women experiencing their first pregnancy or who are of reproductive age form categories of people most susceptible to ambient and ingestible pollutants, they were chosen as the subject group for developing an initial profile of population exposure to such chemicals.

This study was initiated under the Sound Management of Chemicals (SMOC) program of the Commission for Environmental Cooperation (CEC), a body established by the North American Agreement on Environmental Cooperation. The study had two main objectives:

1. To obtain an initial trilateral assessment of exposure to persistent organic pollutants (POPs) and selected metals in expectant primiparous women (EPW) in southern Canada and Mexico, and to present representative data on women of reproductive age who participated in the US National Health and Nutrition Examination Survey (NHANES); and
2. To enhance the capacity of Mexico's analytical facilities to monitor POPs listed in the Stockholm Convention on Persistent Organic Pollutants, as well as selected metals, thereby establishing the basis for the development of compatible, comparable databases of blood biomonitoring results for Canada, Mexico and the United States.

Study design

To obtain exposure data on the desired study population, EPW were recruited from selected study sites across southern Canada (five sites) and Mexico (10 sites). In the United States, women of reproductive age (defined here as 15–44 years of age) were selected from NHANES 2003–2004; pooled samples from

NHANES 2001–2002 for women aged 20–39 years were used for data on polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs).

Blood collected from the study subjects was analyzed for metals (lead, cadmium and mercury in all three countries, plus nickel in Canada and Mexico) and POPs (including 11 from the United Nations Environment Programme Stockholm Convention list, although endrin, dieldrin, and heptachlor epoxide were measured only in the United States), along with γ -hexachlorocyclohexane (γ -HCH or lindane) and β -hexachlorocyclohexane (β -HCH). Pooled samples were analyzed for PCDDs, PCDFs and PCBs, including dioxin-like PCBs. The measurements were carried out in four laboratories: the *Centre de toxicologie du Québec* at the *Institut national de santé publique du Québec* (INSPQ) in Canada, the Centers for Disease Control and Prevention (CDC) in the United States, and in Mexico the *Centro Nacional de Investigación y Capacitación Ambiental* (Cenica) for metals and the *Universidad Autónoma de San Luis Potosí* (UASLP) for POPs. Mexican samples were split and analyzed by INSPQ and by the Mexican laboratories. Organic contaminant levels were expressed on a lipid-weight basis, to account for differences in fasting status and stage of pregnancy among participants. Rigorous quality control/quality assurance procedures were carried out on the laboratory work to ensure the reliability of results.

Mexican and Canadian sample designs were not nationally representative; instead, convenience samples were taken of EPW in selected geographical areas within these two countries. The United States supplied contaminant data for women of childbearing age from NHANES.

Results

Cadmium, lead, total mercury, and nickel (not measured in the United States) among the metals, as well as selected PCB congeners, oxychlordane, β -HCH and *p,p'*-DDE (*p,p'*-dichlorodiphenyl-dichloroethylene) were detected in at least 70 percent of the women in each country. The report presents, by country, the number of samples analyzed, the percent of each chemical detected, with detection limits, and the concentration range and geometric means, including confidence intervals. PCDDs, PCDFs, and co-planar PCBs were measured in composite or pooled samples and the mean results are presented. These

environmental chemicals enter the body via ingestion (generally dietary), inhalation, and dermal absorption. For the general population, the predominant route is through dietary ingestion. The highest concentrations in humans would be expected to be found in areas where these chemicals have been used or produced more recently and in higher quantities. They can, however, also be transported long distances, across borders, by prevailing air currents and thus be found thousands of miles from their sources. POPs tend to be stable in the body and in the environment as well as being lipophilic; hence they bioaccumulate in the fatty and other lipid tissues of humans.

The analyses conducted during the study reveal the predominant PCB congener in all population groups to be PCB 153, followed by 138, 180 and 118. This is in agreement with other biomonitoring studies that have shown PCBs 138 and 153 to be the major PCB congeners in North America and Europe. PCBs are technical mixtures used in a variety of products including transformers and capacitors and were widely used throughout the United States. Other POPs found in a high percentage of the populations included p,p'-DDE, beta-HCH, and oxychlordane. The first of these is the major environmental degradate and human metabolite of p,p'-DDT, which has been widely used in Mexico in agriculture and as a mosquito repellent and insecticide. Beta-HCH is the most stable HCH isomer found in technical hexachlorocyclohexane formulations, which were banned in Mexico more recently than in the United States or in Canada. As expected, cadmium, mercury and lead were detected at low concentrations in a high percentage of the women. Lead exposure can result from gasoline fumes and pottery glazing, among other sources.

Capacity-building

The North American capacity-building initiative was useful in providing insights for necessary biomonitoring improvements. The training and quality-assurance exercises, which took place primarily between the two Mexican laboratories and the Canadian laboratory from 2004 through 2006, were helpful in building analytical capacity in the two Mexican laboratories, and in improving the accuracy and reproducibility of their analytical results. It is important to note that biomonitoring of the general population for environmental contaminants is much different from occupational biomonitoring and especially different from environmental monitoring, both in the matrices of samples and in the concentrations of the analytes, which, in the case of

environmental chemicals, tend to be significantly higher and therefore easier to measure. Future capacity issues include instrumentation of the proper sensitivity to enable the two Mexican laboratories to conduct general population biomonitoring, and for the recruitment and training of key personnel to conduct such studies. The training should include continuing, active participation in external quality-assessment schemes, to allow personnel to demonstrate their proficiency objectively. The assessment schemes should include all the steps of population biomonitoring, from sample procurement, shipping, handling and storage, through analysis and data reporting.

Conclusion

This study has produced an initial North American trilateral profile of human exposure to environmental contaminants, including POPs and selected metals. This preliminary dataset will assist in setting priorities and tracking progress in the management of these chemicals, both domestically and on a broader, North American cooperative basis. This study should not be considered a national biomonitoring program for Mexico or for Canada, since such a program would require a much larger and randomly-selected population sample, similar to what has been done in the United States and is being implemented in Canada. In order for Mexico to plan and conduct a national biomonitoring program, its analytical facilities must be expanded and improved and qualified personnel trained and retained. The success of a nationwide biomonitoring program will depend upon the involvement of key personnel in other several disciplines and institutions, including epidemiologists, statisticians, and chemists. It will also require the necessary administrative support. Only then will it be possible to generate data of sufficient quality for the program to play its role properly as a cornerstone of environmental health policy.

1. Introduction

1.1 Purpose

This study had two general objectives:

1. To obtain an initial trilateral profile of exposure levels to persistent organic pollutants (POPs) and selected metals in a particularly susceptible human population and
2. To enhance the capacity of Mexico to monitor general population concentrations of Stockholm Convention POPs and selected metals, establishing the basis for the development of compatible, comparable databases of blood biomonitoring results for the three countries.

Thus, this study is the first step towards the ultimate goal of providing an integrated data set that could be used to determine priorities and track progress in the management of these chemicals, both domestically and on a broader, North American cooperative basis. The samples in Mexico and Canada were convenience samples collected from expectant primiparous women (EPW), and were analyzed by standard analytical techniques using accepted quality assurance and quality control methodologies. The samples in the United States were collected as part of the National Health and Nutrition Examination Survey (NHANES) to be representative of the United States general population; the data used in the study came from women of reproductive age (15–44 years of age).

1.2 Background

In 1995, in only its second year of existence, the Commission for Environmental Cooperation (CEC), by Council Resolution, initiated a Sound Management of Chemicals (SMOC) program to coordinate trilateral efforts to reduce exposure to substances toxic to human health and the environment in the three countries. Specifically named among these substances were persistent, bioaccumulative, toxic chemicals (PBTs) that may cross international borders, once released to the environment, and affect human populations far from their point of origin.

Transport of PBTs, generally through atmospheric air currents, can also pose an unacceptably high risk to the environment and to ecosystems upon which human life depends. (For the purposes of this report, the term PBTs comprises several classes of substances, including persistent organic pollutants (POPs) and some metals.)

Convenient and dependable access to and dissemination of relevant, reliable and comparable monitoring information and sound interpretive assessments of that information are crucial to the confirmation and quantification of progress on reducing the risk of exposure. Several of the substances are being addressed by both the SMOC program and the Stockholm Convention on POPs. The availability of such information is important for risk analysis, risk assessment, risk management, and the communication of risks to targeted groups and the general population. These assessments will help to determine progress under these initiatives and to identify and explain important emerging issues related to PBTs in the North American environment.

Under the auspices of the CEC SMOC program, an advisory body was created to initiate the study. It was composed of representatives from the appropriate agencies within the participating countries: i.e., the Department of Health (Health Canada) for Canada, the Centers for Disease Control and Prevention (CDC) and the Environmental Protection Agency (EPA) for the United States, and the National Institute of Public Health (*Instituto Nacional de Salud Pública*—INSP), the Secretariat of the Environment and Natural Resources (*Secretaría de Medio Ambiente y Recursos Naturales*—Semarnat) and the National Institute of Ecology (*Instituto Nacional de Ecología*—INE) for Mexico—together with academics and experts who were active in POPs/metals biomonitoring research in North America, as well as representatives of the CEC itself. The participation of these agencies ensured the use of comparable sampling protocols and ensured that the laboratories would produce comparable analytical results. Representatives of the World Bank and the Pan American Health Organization (PAHO) were also invited to participate, upon the granting of funding requests.

2. Study Outline

2.1 Study Framework

The study was established to enhance the capacity of Mexico to biomonitor Stockholm Convention POPs and selected metals, and to enable the country to work with the United States and Canada in establishing the basis for compatible, comparable databases of blood biomonitoring results. The POPs and metals data from Mexico and Canada were obtained by convenience sampling of expectant primiparous women (EPW) in those countries. The data in the United States are those of women of reproductive age who participated in NHANES 2003–2004, as well as of women aged 20–39 years old selected from the pooled NHANES 2001–2002 samples. These quality-assured data will assist in the further development and implementation of effective strategies for the management of POPs and metals. The chemicals measured in at least one of the populations include 11 of the 12 POPs listed in the Stockholm Convention (excluding toxaphene but including β -HCH) and three of the metals (cadmium, lead and mercury) listed in the United Nations Economic Commission for Europe (UNECE) Convention on Long-range Transboundary Air Pollution and its Protocol on Heavy Metals, as well as nickel (of particular interest to Mexico, which until 2002 lacked a management plan for the safe disposal of nickel-containing batteries). In addition, other POPs and metals were measured, although they are not addressed in this report.

The population initially selected for biomonitoring from Canada and Mexico was EPW from 18 to 30 years of age. The women selected were the first pregnant women attending the sampling sites who met the selection criteria (that is, a convenience sample). A search of the literature indicated that there exists an extensive comparative database in Canada for this population, particularly for northern Canada, including accepted protocols and patient-consent models for maternal sampling during routine pre-natal assessments. EPW also provide researchers with a consistent body burden of contaminant levels unaffected by lactation or by *in utero* transfer from mother to a previous infant. Working with pregnant women also enables outreach on

nutrition, the effects of lifestyle and pollutant exposure, and health follow-up to optimize the potential for infant health and development.

The study was originally intended to last 12 months; however, difficulties in obtaining ethics-board approvals from all participating hospitals and institutions and in the recruitment of study subjects meeting the inclusion criteria extended the time required to collect, prepare and analyze the samples. There was also some recruitment difficulty in Mexico because of the young age at which many women have their first child; conversely, in Canada a similar sampling program found many women had not yet had their first child by age 30. Therefore, the participant age range was expanded to include women 14 to 43 years of age.

The study incorporated additional quality-assurance procedures. First, sample collection, storage and analyses were monitored for consistency and quality. Second, chemical analyses of blood samples were conducted by four different laboratories: one in Canada (*Institut national de santé publique du Québec*—INSPQ) which performed both metals and POPs measurements, two in Mexico (Cenica for metals and UASLP for POPs) and one in the United States (CDC) for POPs and metals. Third, analytical results and questionnaire responses were subjected to standardized quality-control tests to ensure comparability and compatibility.

The study specifically involved development of common biomonitoring methodology and capacity-building in Mexico. Capacity-building activities included analytical laboratory training exchanges, translation of guidance and survey questionnaire materials into Spanish, a training exercise regarding medical sampling procedures, and data interpretation and reporting. One of the more important aspects of the study was a quality-assurance/quality-control initiative to ensure that the reporting of analytical methodologies and analytical results were conducted in a consistent and comparable manner. Laboratories were provided with reference samples for which the concentration of a contaminant was known and results of the analyses of these samples were compared to the known values. Any results consistently outside the acceptable range of values resulted in an assessment of the laboratory's procedural methodologies and analytical performance; considerable efforts were directed to locating the sources of the discrepancy and to improving analytical precision and accuracy.

3. Study Design

The study uses a sampling protocol that produces a simple convenience sample of EPW in Canada and Mexico, although the countries used slightly different methods for population selection. In the United States, women of reproductive age were included, whether pregnant or not.

3.1 Population Selection and Recruitment

3.1.1 Inclusion and Exclusion Criteria—Mexico and Canada

For Mexico and Canada, only EPW aged between 14 and 43 years and able to provide informed consent were to be included in this study. Exclusion criteria for this study included any disease that could significantly affect maternal or child health, specifically including pre-eclampsia, diabetes or gestational diabetes, hypertension, epilepsy and endocrine disorders.

3.1.2 Canada

Study subjects were recruited from five southern Canadian cities between December 2005 and August 2007. The cities represent the East Coast (Halifax), the West Coast (Vancouver), Eastern Canada (Hamilton, mainly industrial, and Ottawa, primarily white-collar), and Western Canada (Calgary) (Figure 1). EPW attending their first prenatal visit were approached to participate in this study until 25 women at each site had agreed to enroll in the study. Informed consent was obtained from each woman. The study protocol was reviewed and approved by the research ethics boards of Health Canada and of each of the participating centers.

Figure 1. Sites selected for the study



3.1.3 Mexico

Between November 2005 and February 2006, 25 EPW in the last trimester of pregnancy were recruited from each of ten sites (Figure 1). The recruitments were made in one of the later prenatal visits, at which time the doctor in charge invited the women to participate. If they agreed to participate, the women were directed to the study staff member who explained the study, after which the participants signed a consent form. The study protocol was reviewed and approved by the research, ethics and bio-security commissions of INSP.

3.1.4 United States

For the United States, data come from NHANES, an ongoing national survey of the health, nutritional status and environmental chemical exposures of adults and children.

Although the number of environmental chemicals monitored in NHANES increased from 27 in 1999 to more than 200 in 2003–2004, POPs and metals have been continuously monitored. For this study, data on metals and POPs were obtained from the samples collected from women of reproductive age (15–44 years), including analyses of the samples taken from pregnant women who had participated in NHANES 2003–2004 (the last years for which data were available at the time this report was written) as well as from pooled samples from for women aged 20–39 years taken during NHANES 2001–2002. During NHANES, blood was collected by mobile examination centers.

3.2 Study Implementation and Samples Collected

All blood collection tubes, needles and sample vials were pre-screened for the target metals by the CDC and were provided to each of the participating sites.

3.2.1 Canada

EPW blood was collected in the third trimester, prior to delivery. Samples were collected according to the agreed sampling and laboratory protocols. Blood was collected from the antecubital vein into purple-top, EDTA-containing Vacutainer® tubes (two 7 mL tubes for trace metal measurements and two 10 mL tubes for POPs measurements). Anticoagulated whole blood was stored at 4°C until required for analysis. Plasma, obtained by centrifugation of POPs tubes, was decanted into pre-cleaned vials and stored frozen. Duplicate samples were collected to allow follow-up analyses. All plasma samples were shipped frozen to the analytical laboratory.

3.2.2 Mexico

EPW blood was collected toward the end of the third trimester, according to the agreed sampling and laboratory protocols. Samples were collected by trained field workers coordinated by the National Institute of Public Health. Duplicate samples from Mexico were collected so that analyses could be completed by

one or the other of the national laboratories (Cenica or UASLP) and by the Canadian reference laboratory. Blood collection and storage was carried out as described in section 3.2.1.

3.2.3 United States

Approximately 90 mL of blood were collected from each subject for a variety of clinical chemistry tests, nutritional status tests and measurements of environmental chemicals in either the whole blood (metals) or harvested serum (POPs, among others). The serum and whole blood were sent frozen on dry ice to the CDC laboratory and stored at no more than -70°C until analysis.

3.2.4 Dioxins and Furans: Pooled Samples

To avoid drawing large volumes of blood from the EPW and to limit analytical costs, it was decided at the outset of the study that pooled plasma samples would be analyzed for PCDDs, PCDFs and both dioxin-like and non-dioxin-like PCBs. From these data, dioxin toxic equivalent (TEQ) concentrations and total PCB concentrations were calculated. In Mexico two composite plasma samples were prepared for each of the ten sites, for a total of 20 composite samples, while for the Canadian samples, three composites were prepared from each of three sites, two composites from one site and one sample for one other site, for a total of 12 composite samples. In the United States, seven pooled serum samples with balanced volume from women between 20 and 39 years of age participating in NHANES 2001–2002, who may or may not have been pregnant, were analyzed for PCDDs, PCDFs and dioxin-like PCBs, and TEQs were calculated.

3.3 Analytical Issues

3.3.1 Analytes and Matrices

All metal concentrations are reported on a whole-blood volume basis. The POPs data in serum and plasma are reported on a lipid-adjusted weight basis. The total lipid concentration of each sample was calculated by summing the measured concentrations of plasma lipid components. All lipid components were measured by standard clinical chemistry enzymatic methods.

INSPQ measured four components: total cholesterol (TC), free cholesterol (FC), triglycerides (TG), and phospholipids (PL), then calculated the total lipid (TL) concentration as:

$$TL = 1.677 * (TC-FC) + FC + TG + PL \text{ (Akins et al. 1989).}$$

CDC measured total cholesterol and triglycerides and calculated total lipids as:

$$TL = 2.27 * TC + TG + 0.623 \text{ (Philips et al. 1989).}$$

Both formulas yield equivalent total lipid results (Bernert 2007).

Even though blood samples were taken from both pregnant and non-pregnant women, studies have shown that this is not a major issue if the POPs concentrations are adjusted for lipid content.

The following analytes were measured:

Metals: Mexican and Canadian whole-blood samples were analyzed for lead, mercury (total and inorganic), cadmium, cobalt, nickel, selenium, thallium and tin. The US whole blood samples were analyzed for lead, mercury (total and inorganic), and cadmium.

POPs: A total of 60 POPs were measured, including 11 non-dioxin-like PCB congeners and 15 organochlorine pesticides, metabolites, or byproducts measured in the same individual blood samples as the metals, as well as the 17 2,3,7,8-substituted PCDDs and PCDFs and 12 dioxin-like PCBs measured in composite plasma samples (**Tables 1** and **2**). The composite samples were also analyzed for a total of 35 PCB congeners in order to calculate total PCB concentrations. It should be noted that most of the mono-ortho substituted PCBs are included in the total PCB concentrations, but the coplanar PCBs are not, because of their relatively low concentrations and because they are measured analytically with the PCDDs and PCDFs.

Table 1. Stockholm Convention POPs measured in plasma (Canada, Mexico) and serum (US–NHANES)

Parent Chemical or Chemical Class	Analyte(s)
Aldrin	Aldrin, Dieldrin*
Endrin	Endrin*
Dieldrin	Dieldrin*
Chlordane	Oxychlordane, cis-Chlordane**, trans-Chlordane**, cis-

	Nonachlor**, trans-Nonachlor
Heptachlor	Heptachlorepoxyde*
DDT	p,p'-DDT, p,p'-DDE (p,p'-Dichlorodiphenyldichloroethylene)
Mirex	Mirex
Hexachlorobenzene (HCB)	Hexachlorobenzene
Polychlorinated biphenyls (PCBs)	Congeners: Non-dioxin like: 28, 52, 99, 101, 128, 138, 153, 170, 180, 183, 187. Dioxin-like mono-ortho chlorinated: 105, 114, 118, 123, 156, 157, 167, 189. Dioxin-like coplanar+: 77, 81, 126, 169
Polychlorinated dibenzo-p-dioxins+	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, OCDD
Polychlorinated dibenzofurans+	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, 12,3,7,8,9- HxCDF, 2,3,4,6,7,8- HxCDF, 1,2,3,4,6,7,8- HpCDF, 1,2,3,4,7,8,9- HpCDF, OCDF

* not measured in samples from Canada and Mexico

** not measured in samples from the United States

+ measured in sample pools from Canada, Mexico and the United States

Table 2. Other POPs measured in plasma (Canada, Mexico) and serum (US–NHANES)

Parent Chemical	Analyte(s)
Technical hexachlorocyclohexane	α-HCH**, β-HCH, γ-HCH
Polybrominated biphenyls	Polybrominated biphenyl 153

** not measured in samples from the United States

3.3.2 Analytical Methods

3.3.2.1 Canada and Mexico

Biological samples from Canada and Mexico were analyzed by the INSPQ laboratory. Samples from Mexico for some analytes were also measured in-country, but the results from the Canadian reference laboratory were utilized for the data analyses for both countries, with the exception of the composite samples, which were analyzed for PCDDs, PCDFs, and PCBs by the CDC laboratory. Metals were measured by either inductively-coupled mass spectrometry (lead and cadmium) or cold-vapor atomic absorption (mercury) (Butler-Walker 2006). Chlorinated pesticides and PCBs were measured using high-resolution gas chromatography/low-resolution mass spectroscopy (GC-MS) using an in-house, ISO-17025 accredited method adapted from Mes (1990). Plasma samples (2 mL) were extracted on a solid phase extraction (SPE) column. The extracts were purified on a Florisil column, concentrated to a final volume of 100 µL, and analyzed by gas chromatography-mass spectrometry using electronic impact ionization (EI).

3.3.2.2 United States

Whole blood samples (50 µL) were analyzed for mercury (total), lead and cadmium by inductively-coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) with a Meinhard nebulizer, a cyclonic spray chamber and an AS-93 autosampler. In this method blood samples are diluted with ≥ 18 M-ohm-cm water and diluent, containing 1 percent v/v tetramethylammonium hydroxide (TMAH), 0.5 percent disodium ethylenediamine tetraacetate (EDTA), 10 percent ethyl alcohol, 0.05 percent Triton X-100® and gold (100 µg/L). Gold (Au) is added to reduce intrinsic mercury memory effects. The internal standards used were rhodium (Rh) (5 µg/L) for cadmium and bismuth (Bi) (5 µg/L) for mercury and lead. The samples were prepared with the ratio of Sample : Water : Diluent = 1:1:48 (Nixon et al. 1999).

Serum/plasma samples (1 mL) were analyzed for POPs by extracting through solid phase extraction, then detecting and measuring by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (Barr et al. 2003). PCDDs, PCDFs and PCBs were measured in the composite samples by the same method for all three countries (Patterson et al. 1987, 1990). The limit of detection (LOD) for POPs varied with each sample, based on several factors, including volume of serum used and percent recovery of the analyte.

3.3.3 Quality-Assurance and Quality-Control Procedures

3.3.3.1 Canada and Mexico

For metals, internal quality control was accomplished through the use of reference material from the INSPQ's Interlaboratory comparison program (*Programme de comparaisons interlaboratoires*, or PCI) external quality assessment scheme (EQAS) (Weber 1996). These materials were analyzed several times within each analytical run (after the calibration curve, after every 10 samples, and at the end). Results were plotted on control charts having predetermined acceptability limits and were required to comply with the Westgard rules for run results to be accepted (Westgard 2001). Accuracy was verified by participation in several EQAS from Canada, Germany, and the United States (INSPQ 2007, Schaller 2001, Stanton 1993).

For POPs, precision was monitored through the use of in-house reference materials, analyzed with each analytical run, using the same procedure as for metals. Routine checks of accuracy were made using certified reference serum from the US National

Institute of Science and Technology. Also, accuracy was verified periodically through participation in two EQAS. The LOD for POPs varied between 0.005 and 0.09 µg/L, depending on the analyte.

3.3.3.2 United States

For metals in blood, CDC used both low- and high-level bench quality-control materials (blood mercury, lead and cadmium), prepared in the CDC laboratory, as well as two levels of “blind” QC materials. Bench and blind QC materials consist of 1.8 mL of blood material in 2 mL cryo-vials stored at $\leq -70^{\circ}\text{C}$ until use. Both the bench and blind QC materials were characterized in 40+ analytical runs. In addition, National Institutes of Standards and Technologies (NIST) Standard Reference Material (SRM) 966 and 955b were routinely used for method-accuracy determinations. Analytical accuracy was also verified by participation in several EQAS programs from INSPQ, the New York State Health Department and the Wisconsin State Health Department. To evaluate the validity of results, QC data within each analytical run were compared to predetermined control limits using the Westgard rules (Westgard 2002).

For POPs, pooled serum was used for QC materials. No further filtration or preparation of the serum pools was performed prior to their use. The pooled serum was aliquoted into vials, capped and stored at -20°C . All QC concentration levels were characterized to determine the mean concentrations and the 95th (1.96σ) and 99th (2.58σ) control limits by consecutive analysis of 20 samples of each QC level. QC data within each analytical run were compared to the control limits to evaluate the validity of analyses using the Westgard rules, as above.

3.3.4 Limits of Detection Issues

3.3.4.1 Determination of Limits of Detection

In the United States, the analytical method LOD for each analyte within the method was calculated as $3S_0$, where S_0 is the estimated standard deviation of measured concentration values as the concentration approaches zero. With this technique, S_0 is an extrapolated value equivalent to the y-intercept of a regression line from the plot of the standard deviations of the measured concentrations versus their nominal concentration values (Taylor 1987, 75–93). Alternatively, for analytes having a method blank, the LOD was calculated as $3S_b$, where S_b is the estimated standard deviation of blank values (Keith 1991, 93–

119). The LOD is adjusted according to the analyte recovery in each individual sample. LOD calculations are determined based on 20+ analytical runs. The LOD calculations for the blood metals were determined using multiple analysts and instruments on 40+ analytical runs.

In Canada, a similar approach to LOD determination was used by the INSPQ laboratory. For analytical signals in the form of peaks (e.g., chromatographic output), an initial LOD was estimated as:

$$\text{LOD}_{\text{est}} = \text{sample concentration} / ((\text{observed signal/noise ratio})/3).$$

For continuous or averaged analytical signals (e.g., ICP-MS), an initial LOD was estimated as three times the standard deviation of the blank. A real or matrix-matched spiked sample having a concentration between 5 and 7 times the LOD_{est} was then analyzed 10 times. The LOD was defined as the standard deviation of these 10 measurements (S_0) multiplied by 3.

3.3.4.2 Comparison of Limits of Detection between the United States and Canada

As shown in **Tables 3** and **4**, the LOD for selected POPs and metals results produced by CDC and INSPQ are not identical, and in some instances are substantially different.

4. Statistical Analysis

The Canadian EPW population was divided into two subgroups (Canada-by-birth and Canada-immigrant). This was not done for the US women of reproductive age. For the Mexico data the samples were considered to be homogenous, in that all the EPW were native-born.

Summary statistics, including sample size, LOD and percent of observations above LOD were reported for all contaminants in each group. The range, geometric mean (GM) and corresponding 95 percent confidence interval were evaluated for each contaminant, except γ -HCH. For γ -HCH in Mexico, only the range was reported, given the low frequency of detection. The GM and corresponding 95 percent confidence interval were reported in place of the arithmetic mean and corresponding 95 percent confidence interval, because most of the contaminants were log-normally distributed, as shown by the Anderson-Darling test for normality. Values below the LOD were imputed with $\frac{1}{2}$ the LOD.

5. Results

Table 3 reports summary statistics for 12 contaminants in the Mexican and Canadian populations and **Table 4** reports similar data for the US population (with the exception of nickel). For the data analysis in **Table 3**, the more comprehensive set of results for Canada and Mexico from the Canadian reference laboratory were used.

Table 3. Descriptive statistics and summary results for 12 contaminants in EPW in Canada and Mexico

Contaminant	Data Provenance	N	% Detects	Detection Limit (DL)	Range	Geometric Mean (95% CI)
Cadmium (µg/L)	Canada-immigrant	16	100	0.05	(0.25–2.02)	0.59 (0.42–0.83)
	Canada-by-birth	77	100	0.05	(0.16–4.95)	0.46 (0.38–0.55)
	Mexico	233	100	0.05	(0.16–1.69)	0.36 (0.34–0.37)
Lead (µg/L)	Canada-immigrant	16	100	0.21	(3.52–33.15)	7.84 (5.65–10.86)
	Canada-by-birth	77	100	0.21	(2.69–12.02)	5.72 (5.34–6.13)
	Mexico	233	100	0.21	(5.59–227.93)	24.87 (22.87–27.04)
Nickel (µg/L)	Canada-immigrant	16	100	0.35	(0.59–4.05)	2.49 (1.95–3.18)
	Canada-by-birth	77	100	0.35	(0.18–5.58)	2.14 (1.91–2.40)
	Mexico	233	100	0.35	(1.29–6.46)	3.23 (3.13–3.33)
Total Mercury (µg/L)	Canada-immigrant	16	94	0.1	(0.05–4.21)	0.88 (0.55–1.41)
	Canada-by-birth	77	92	0.1	(0.05–2.81)	0.40 (0.32–0.50)
	Mexico	233	97	0.1	(0.05–18.05)	0.86 (0.75–0.97)
PCB 118 (µg/kg Lipid)	Canada-immigrant	20	95	1.33	(0.81–7.27)	2.49 (2.03–3.06)
	Canada-by-birth	103	89	1.29	(0.63–12.64)	2.15 (1.92–2.40)
	Mexico	240	46	1.29	(0.42–57.75)	1.22 (1.11–1.33)
PCB 138 (µg/kg Lipid)	Canada-immigrant	20	100	1.33	(2.17–22.00)	6.00 (4.71–7.65)
	Canada-by-birth	102	100	1.29	(1.03–20.78)	3.69 (3.31–4.11)
	Mexico	240	82	1.29	(0.52–50.70)	2.38 (2.15–2.62)
PCB 153 (µg/kg Lipid)	Canada-immigrant	20	100	1.33	(4.35–49.00)	11.08 (8.63–14.22)
	Canada-by-birth	103	100	1.29	(1.27–19.54)	5.67 (5.07–6.34)
	Mexico	240	93	1.29	(0.52–72.50)	3.64 (3.30–4.01)
PCB 180 (µg/kg Lipid)	Canada-immigrant	20	100	1.33	(1.70–32.00)	7.90 (5.93–10.53)
	Canada-by-birth	103	89	1.29	(0.52–85.54)	3.44 (2.88–4.11)
	Mexico	240	74	1.29	(0.51–43.62)	2.08 (1.87–2.30)
Oxychlordane	Canada-immigrant	20	100	0.67	(1.09–11.90)	2.85 (2.17–3.75)

Contaminant	Data Provenance	N	% Detects	Detection Limit (DL)	Range	Geometric Mean (95% CI)
(µg/kg Lipid)	Canada-by-birth	102	100	0.65	(0.83–10.00)	2.08 (1.90–2.29)
	Mexico	240	89	0.65	(0.30–15.05)	1.62 (1.50–1.76)
β-HCH (µg/kg Lipid)	Canada-immigrant	20	95	1.33	(0.88–988.64)	7.74 (3.83–15.63)
	Canada-by-birth	103	84	1.29	(0.42–25.64)	2.12 (1.85–2.43)
	Mexico	240	98	1.29	(0.63–209.68)	8.34 (7.31–9.53)
γ-HCH (µg/kg Lipid)	Canada-immigrant	19	0	1.33	ND	ND
	Canada-by-birth	103	0	1.29	ND	ND
	Mexico	240	2	1.29	(0.33–6.90)	ND
p,p'-DDE (µg/kg Lipid)	Canada-immigrant	20	100	11.97	(33.33–1704.55)	162.17 (107.09–245.58)
	Canada-by-birth	103	100	11.62	(14.49–294.87)	52.77 (47.91–58.12)
	Mexico	240	100	11.62	(46.51–19753.09)	335.68 (295.08–381.86)

Table 4. Descriptive statistics and summary results for 11 contaminants for reproductive age women 15–44 years old in the NHANES survey, 2003–2004

Contaminant	N	Weighted % Detects	Detection Limit (DL)	Range	Weighted Geometric Mean (95% CI)
Cadmium (µg/L)	1649	70.9	0.14	(0.1–7.4)	0.32 (0.30–0.35)
Lead (µg/L)	1649	92.8	2.8	(2–239)	9.38 (8.66–10.16)
Total Mercury (µg/L)	1649	84.6	0.2	(0.1–30.7)	0.79 (0.69–0.92)
PCB 118 (µg/kg Lipid)	490	100	0.6	(0.95–40.36)	4.45 (3.89–5.1)
PCB 138 (µg/kg Lipid)	491	100	0.4	(1.39–70.17)	8.89 (7.83–10.09)
PCB 153 (µg/kg Lipid)	490	100	1.1	(1.6–90.32)	10.95 (9.73–12.32)
PCB 180 (µg/kg Lipid)	491	99.6	0.4	(0.06–82.56)	7.26 (6.45–8.18)
Oxychlordane (µg/kg Lipid)	500	63.6	7.8	(1.06–36.4)	5.83 (5.31–6.41)
β-HCH (µg/kg Lipid)	498	55.5	7.8		ND
γ-HCH (µg/kg Lipid)	499	0.6	7.8*		ND
p,p'-DDE (µg/kg Lipid)	495	99.8	7.8*	(16.4–15100)	150.42 (120.27–188.13)

ND (non-detect) means further calculations were not applicable due to the low percent of observations being detected (<10%).

Table 5 presents the data on age of the four groups included in this study. The youngest women were found in Mexico, while the oldest were found in the Canadian populations. Because many of the POPs bioaccumulate in the lipids of the body (including blood lipids), older populations could now be experiencing similar degrees of exposure as younger populations, yet have higher blood POPs concentrations because of bioaccumulation. For the United States data, different one-third subsets were used for measuring PCBs and for measuring organochlorine pesticides (OCPs); therefore, the number of women of reproductive age in the PCBs and OCPs groups differ slightly.

Table 5. Summary of age in each group across all contaminants

Group	N	Mean Age	Range
Canada-immigrant	20	33.0	(25–41)
Canada-by-birth	101	28.6	(18–40)
Mexico	240	20.8	(15–33)
United States (NHANES 2003–2004)—Metals*	1649	26.1	(15–44)
United States (NHANES 2003–2004)—PCBs [†]	491	26.4	(15–44)
United States (NHANES 2003–2004)—OCPs [‡]	500	26.1	(15–44)

* NHANES women aged 15–44 were selected from the full sample for metals.

† NHANES women aged 15–44 were selected from a one-third subsample for polychlorinated biphenyls (PCBs).

‡ NHANES women aged 15–44 were selected from a different one-third subsample for organochlorine pesticides (OCPs).

6. Discussion

Data for POPs are presented in this report on a lipid-adjusted basis (i.e., $\mu\text{g/kg}$ lipid) while the metals are presented on a whole blood basis ($\mu\text{g/L}$). As lipid concentrations increase throughout pregnancy and in response to meals, comparisons of POPs on a lipid-weight basis would be more reasonable.

6.1 Concentrations of Persistent Organic Pollutants

Results for the four most highly-detected PCB congeners (118, 138, 153, 180) are presented in **Tables 3** and **4**. The predominant PCB congener in all population groups is PCB 153, followed by 138, 180 and 118. PCBs 138 and 153 have been shown to be the major PCB congeners in other North American and European biomonitoring studies (Needham et al. 2005, Becker et al. 2002). PCBs are technical mixtures used in a variety of products including transformers and capacitors and were widely used throughout the United States. Other POPs found in a high percentage of the populations included p,p'-DDE, Beta-HCH, and oxychlordane. p,p'-DDE is the major environmental degradate and human metabolite of p,p'-DDT, which has been widely used in Mexico in agriculture and as a mosquito repellant and insecticide. Beta-HCH is the most stable HCH isomer found in technical hexachlorocyclohexane formulations, which were banned in Mexico more recently than in the United States or in Canada. As expected, cadmium, mercury and lead were detected at low concentrations in a high percentage of the women. Lead exposure can result from gasoline fumes and pottery glazing, among other sources.

6.2 Concentrations of Dioxins in Pooled Blood

Table 6 shows the concentrations for lipid-weight adjusted arithmetic means and ranges for the total dioxin TEQs as well as TEQs for PCDDs, PCDFs and dioxin-like PCBs (coplanar PCBs and mono-ortho substituted PCBs) from pooled plasma samples of EPW from Mexico and Canada, and from pooled serum samples of women aged 20–39 from the United States.

Table 6. Toxic Equivalent Concentrations¹ of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, co-planar PCBs, mono-ortho PCBs and Total TEQs in pooled serum

Country	PCDDs		PCDFs		Coplanar PCBs		Mono-ortho PCBs		TOTAL TEQ	
	Mean*	Range	Mean*	Range	Mean*	Range	Mean*	Range	Mean*	Range
Mexico	4.3	2.9–15	1.3	1.0–1.6	1.1	0.69–1.7	0.09	0.04–0.15	6.8	5.3–18
Mexico (outlier removed)	3.8	2.9–5.9	1.3	1.0–1.6	1.1	0.69–1.7	0.09	0.04–0.15	6.3	5.3–9.4
Canada	3.3	2.6–5.6	1.4	1.2–2.0	1.2	0.76–1.4	0.17	0.12–0.23	6.1	5.1–9.2
United States (NHANES 2001–2002)	6.7	4.1–9.0	1.8	1.4–2.3	1.7	1.2–2.3	0.31	0.17–0.41	10.5	8.1–14

1. All results presented as 2,3,7,8 TCDD TEQs.

* Arithmetic mean

The World Health Organization's 2005 Toxic Equivalency Factors (TEFs) were used to calculate the TEQs (Van der Berg et al. 2006). The TEQs were calculated by multiplying the individual concentration (ng/kg lipid) of each of the PCDDs, PCDFs and dioxin-like PCB congeners by that chemical's TEF to provide the TEQ for each of these chemicals; these TEQs were summed to provide class-specific TEQs (e.g., for PCDDs) and for total TEQs.

Table 7 shows arithmetic mean concentrations and ranges of the sum of 35 PCB congeners (total PCBs) for the pooled samples from Mexico, Canada and the United States. For calculating total PCBs, if a given congener was not detected, its concentration was imputed to be its LOD/2.

Table 7. Sums of PCB concentrations in serum pools

	Total PCB (µg /kg lipid)	
	Mean*	Range
Mexico	36.6	18.3–59.0
Canada	50.7	25.5–95.3
United States (NHANES 2001–2002)	83.2	50.5–109

* Arithmetic mean

Arithmetic means were computed instead of geometric means for all of the pooled data because of unequal numbers of samples per pool and unequal volumes for some pools in the Canada data. Also, the Mexican data consisted of only two pools from each site, which limited the ability to estimate the variation among the

pools within each site. This variation is needed in order to convert pooled sample results to geometric mean (GM) estimates, although these could have been calculated from the NHANES pooled samples, but then any comparisons with the Canada and Mexico data would not be appropriate.

By using arithmetic means rather than geometric means, the results are positively biased, and this bias may vary with concentration if the among-subject variances of the compounds of interest tend to vary with increasing concentration (Caudill et al. 2007). As a result, caution is required in comparing arithmetic mean estimates across sites or across countries. Because only pooled data are available, providing no measure of individual variability, only descriptive results can be presented. Like the other POPs data presented in this report, data in **Tables 6 and 7** are not age-adjusted.

7. Capacity Building

7.1 Training and Quality Assurance Exercises

7.1.1 Introduction

An important component of the capacity building exercise was to ensure the accuracy and comparability of results produced by the Mexican laboratories involved in the study. These laboratories are those of the UASLP for the measurement of POPs and Cenica for the measurement of metals. To this end, an Operational Plan for Quality Assurance/Quality Control was submitted by the reference laboratory INSPQ in 2004. The key features of this plan were:

- Training of UASLP staff at the INSPQ lab for POPs measurements and familiarization with QA/QC procedures.
- Method validation and external quality assurance (EQA) exercises for both laboratories, in which the target laboratories (Cenica and UASLP) were sent representative proficiency-testing material (blood and plasma samples) containing the required analytes.

In the case of Cenica, these analytes were metals (lead, cadmium and mercury) in blood. For UASLP, proficiency testing materials consisted of plasma samples containing various PCB congeners and organochlorine pesticides. Chemical analyses for each sample were conducted by the different laboratories: INSPQ for metals and all POPs except dioxins, Cenica for metals and UASLP for POPs. All were subjected to reference standard calibration verification. Dioxin and dioxin-like compounds were all analyzed at the CDC laboratory.

Both Mexican laboratories were invited to participate in the appropriate INSPQ external quality assurance schemes: UASLP in the AMAP Ring Test for POPs, and Cenica in the PCI for metals.

7.1.2 Results

Training of UASLP staff (two members) took place 19–30 April 2004, at INSPQ's toxicology laboratory in Quebec City. The training focused on methodology for the measurement of POPs in blood, including sample preparation, purification and instrumental analysis. The trainees were tested at the conclusion of the training period and demonstrated proficiency in conducting the analyses.

For each laboratory, two quality-assurance exercises were held, one in 2004, the other in 2006. Several more exercises had been planned; however, major difficulties were encountered in cross-border shipments to Mexico, which require specific import permits for each shipment of biological material. Nevertheless, the two exercises were beneficial in uncovering problems and documenting improvements in the quality of results from both laboratories.

Both laboratories were repeatedly encouraged to enroll in the relevant INSPQ external quality-assurance schemes, resulting in participation in mid-2006. Again, forwarding of proficiency-testing samples was beset by delays and rejections of shipments by Customs officials unfamiliar with this type of effort. Cenica completed only one round; UASLP completed none.

Analytical results were compared by using split or co-located samples analyzed in Mexico laboratories and INSPQ. CDC supplied statistical expertise to compare results provided by INSPQ and Cenica for metals and UASLP for POPs. Results show that UASLP had a preponderance of non-detectable values for the organochlorine chemicals. Therefore comparability analyses were performed solely on p,p'-DDE.

Results are based on 219 pairs of data and showed that the DDE (log scale) data obtained by UASLP tends to give a higher reading, by between 0.03 and 0.12. However, only 5 percent of log DDE ratios (difference) exceeded limits of agreement. A paired t-test was used to compare means on the log scale from both laboratories; the result showed that UASLP had significantly higher DDE means than INSPQ ($p < 0.01$).

7.2 Recommendations on Improving Proficiency

It is worth noting the potential for induced measurement errors based on laboratory proficiencies and prior experience with the testing of particular sample media (in this case, either whole blood or blood serum). The facilities at Cenica are routinely

involved in the measurement of selected toxicants, including metals, in environmental samples of air, water, soils and sediments. Measurement of these same toxicants in blood or blood serum presents a unique set of challenges for which the facility and the analysts themselves may not be prepared. Blood and blood serum are potentially complex matrices—quite different from the matrices expected in environmental samples. The challenges they represent were exemplified by the first round QA/QC testing in 2004. The laboratory showed good agreement with reference samples when the anticipated toxicant values were provided prior to the analysis. When the anticipated results were unknown, however, the measured values varied considerably from the reference values. This is not uncommon in analytical laboratory round-robin QA/QC proficiency testing. Subsequent to this validation exercise, discussions were held with the laboratory personnel and the analytical protocol expert contracted by the CEC. The discussions resulted in significant improvement in the analytical results of the laboratory and a much-enhanced appreciation of the QA/QC aspects of good laboratory practices. Later rounds of analysis did validate the results and contributed to a significant improvement in both precision and accuracy.

In a similar vein, UASLP, in undertaking the POPs measurements on behalf of INSP, had anticipated that analyte concentrations would be in the range of previous work where levels associated with crop- or vector-control application had been determined. In fact, it is now apparent that very low levels near the LOD of state-of-the-art methodologies and equipment would be encountered. The reasons for this include the banning of DDT and lindane, as well as the general trend toward reduced application of organochlorine pesticides. In the past, UASLP facilities had reported results indicating higher levels of exposure. This study, in contrast, looked at a national distribution of samples, some in areas where pesticide residues were likely to be low. As a result, the comparatively higher LOD of the UASLP laboratories caused many “non-detect” entries to be recorded, as shown in **Table 8**.

Table 8: Comparison of LOD for three organic analytes

Analyte	Laboratory	
	UASLP	INSPQ
DDT (µg/L)	0.55	0.05
γ-HCH (µg/L)	0.72	0.01
HCB (µg/L)	0.11	0.04

Results generally improved significantly with subsequent training and analytical testing exercises, thereby underlining the need for laboratory accreditation as well as enrollment and participation in interlaboratory quality-assurance programs or schemes (EQAS). National accreditation bodies able to operate according to ISO 17025, the appropriate standard for analytical laboratories, exist in all North American countries. EQAS, generally including technical assistance, are available through such organizations as the INSPQ external quality-assurance schemes described above, the CDC's Lead and Multi-element Proficiency (LAMP) Program (CDC 2008a) and the German External Quality-Assurance Scheme for analyses of biological material (University of Erlangen-Nuremberg 2008).

It is recommended that an initiative be developed to overcome border obstacles to the transfer of biological proficiency testing samples into Mexico. It is also recommended that a continental laboratory testing and verification initiative be coordinated.

8. Conclusions

The two main purposes of this study were to obtain an initial profile of population exposure to POPs and selected metals and to enhance the capacity of Mexico to monitor Stockholm Convention POPs and selected toxic metals, thus enabling Mexico to work with the United States and Canada in developing compatible, comparable databases of blood biomonitoring results.

8.1 Population Exposure Assessment

This study has shown the feasibility of developing comparable protocols and assessing contaminant levels in populations from Canada, Mexico and the United States. This preliminary dataset can be used to assist in setting priorities for and tracking progress in management of the selected toxicants both domestically and on a broader cooperative basis within North America. This should not be considered a national biomonitoring program for Mexico or Canada, since such a program would require a much larger and randomly-selected population sample, as has been done in the United States and is underway in Canada. The US NHANES program involves collecting health information on 8,000 individuals and measuring contaminant levels in a random one-third subset of about 2,500 individuals every two years (CDC 2008b), while the Canadian Health Measures Survey will measure environmental contaminant levels in approximately 5,000 individuals in representative communities across the country in 2008–2009 (StatCan 2008). A national biomonitoring program for Mexico which could address levels of contaminants in a range of age, gender and ethnic groups would be useful for further comparative work with the United States, Canada and other countries.

While the statistical representativeness of this study was constrained by the small sample sizes, it is apparent that further work should be undertaken to account more precisely for the probable sources of the contaminants, and to evaluate these and any subsequent study results with respect to exposure routes.

8.2 Capacity Building

The capacity building component of the study has been useful in providing insights for improvements necessary to operations on a trilateral basis within North America. The training and quality-assurance exercises between 2004 and 2006 were helpful in building analytical capacity in the two Mexican laboratories, and in improving the accuracy and reproducibility of their analytical results. It is important that the Mexican laboratories continue to participate actively in external quality-assessment schemes, to ensure that they can objectively demonstrate their proficiency.

A crucial difficulty that must be overcome for successful participation concerns the importation of biological proficiency testing samples into Mexico, an issue that appears to be unique to that country. In none of the other 32 countries participating in the INSPQ external quality-assessment schemes have problems been encountered in sending biological proficiency testing samples across borders to recognized laboratories. The continued cooperation of Mexican authorities will be essential in achieving reliable cross-border transmission of proficiency-testing material.

Trilateral efforts such as this one also reveal the importance of rigorous data management. Best practices of data-management procedures should be implemented where lacking, and rigorously applied everywhere. There is a need to develop and use data standards, referring to documented procedures to ensure that data are clearly understood and collected by uniform methodologies. Roles and responsibilities for data management must be established, implemented and documented. The development of detailed agreements specifying who is responsible for development of standards, collection, storage and maintenance of data, protocols for their access and use, and the systematization of quality-assurance procedures.

8.3 Recommendations for Future Work

Mexico

This trinational study has allowed description of concentrations of several environmental contaminants in maternal blood within Mexico and, for the first time, made them in the context of the other two North American countries. This is a significant step forward in regional biomonitoring. However, it is important to point out that some of the study's objectives were not fully

attained. One of these is the creation and improvement of analytical capabilities, including equipment, facilities, and trained personnel within Mexico, to enable the implementation of a permanent human biomonitoring system. From this study we conclude that the Mexican government needs to invest in establishing these analytical capabilities, perhaps with resources from the CEC and other relevant international funding institutions. It is important to include not only the laboratories that participated in this study, but also regional laboratories within the areas where long-range human biomonitoring of POPs will be carried out. Essential components of these enhanced analytical capabilities in Mexico include participation in internationally recognized quality-assurance/quality-control programs and round-robin testing.

In Mexico, long-range monitoring of POPs is part of the National Monitoring and Environmental Evaluation Program (*Programa Nacional de Monitoreo y Evaluación de Sustancias Tóxicas, Persistentes y Bioacumulables* Proname), which includes a biological/human biomonitoring component. One of the objectives of Proname is to assess contaminant concentrations and monitor trends of these contaminants in vulnerable population groups.

The success of Proname hinges on the participation of key institutions. We recommend that the following institutions be included: the National Institute of Public Health (INSP) that coordinated this first effort in the country, the National Institute of Ecology (INE), the National Center for Environmental Research and Training (Cenica), the Ministry of Health, represented by the Federal Commission for Risk Protection (Cofepris), which also includes the Commission for Analytical Control and Extension of Coverage (CCAYAC), the network of laboratories within the Ministry of Health.

We also recommend follow-up human biomonitoring in those cities where the highest POPs levels (including metals such as mercury and lead) were encountered in this initial study, regardless of the relevance to Proname.

Canada, Mexico and the United States

Both Canada in the Canadian Health Measures Survey (CHMS) and the United States in the National Health and Nutrition Examination Survey (NHANES) have national human biomonitoring programs directly relevant to the proposed ongoing biomonitoring in Mexico. Canada and the United States

need to continue to provide population sampling expertise and analytical support for the follow-up studies proposed by Mexico. Canada and the United States will be able to continue to collaborate with Mexico on trinational comparisons of other relevant population subgroups, such as children. Close collaboration on study design of new biomonitoring programs in Mexico must take place to ensure comparability of the population groups studied and the resulting data.

Chemicals not included in this study, such as brominated flame retardants and perfluorinated chemicals, are being measured in the CHMS and NHANES. Only limited monitoring data for these chemicals are available in Mexico. Further sampling and analytical capacity building for human biomonitoring in Mexico must include these chemicals, as they are likely to be considered for addition to various international treaties to which Mexico, Canada and the United States are or may become signatories.

Quality assurance and quality control and participation in international round-robins are essential components in the production of quality data. Canada and the United States must continue to participate with Mexico in the development and assessment of the QA/QC information developed under the proposed monitoring in Mexico. Canada and the United States should also provide analytical training, reference samples and access to various international analytical round-robins as part of this trinational biomonitoring program.

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