Synopsis of Research

Conducted under the 2005-2006
Northern Contaminants Program

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Foreword

This report provides a summary of the progress to date of research and monitioring studies on contaminants in northern Canada, and related education, communications and policy activities that were conducted in 2005-2006 under the auspices of the Northern Contaminants Program (NCP). The projects cover all aspects of northern contaminants issues, as outlined in the NCP blueprints, including human health, monitoring the health of Arctic peoples and ecosystems and the effectiveness of international controls (abiotic monitoring and modeling, and biotic monitoring), education and communications, international policy and program management.

These projects were evaluated as proposals, by external peer reviewers, technical review teams, a social/cultural review team, territorial/regional contaminants committees and the NCP Management Committee to ensure that they support the overall Northern Contaminants Program objectives.

Further information about the Northern Contaminants Program is available on the NCP website at www.ainc-inac.gc.ca/ncp.

Préface

Ce rapport résume l'avancement de recherches et d'études de surveillance portant sur les contaminants dans le Nord canadien, ainsi que d'activités connexes au sujet de l'éducation , de la communication et de la politique qui ont eu lieu en l'année 2005-2006. Ces etudes et activités ont été menées dans le cadre du Programme de lutte contre les contaminants dans le Nord (PLCN). Ces projets, tells que décrit dans les plans directeurs lies au programme, représentent tous les aspects portent sur les contaminants, incluant la santéhumaine, la surveillance de la santé des habitants et des ecosystems de l'Arctique et de l'efficancité des measures de contrôle internationales (surveillance et modélisation milieux abiotiques, et surveillance-milieux biotiques), l'éducation et la communication , la politique internationale et al gestion des programmes.

Ces projects on été examines par des pairs, des comités d'examen technique, un comité d' examen social et culturel, les comités territoriaux/régionaux sur les contaminants environmentaux, et le comité de gestion de la PLCN afin de s'assurer qu'ils respondent à l'ensemble des objectifs du programme de lutte contre les contaminants dans le Nord.

Pour de plus amples renseignements au sujet du programme de lutte contre les contaminants dans le Nord, visitez le site Web du PLCN au www.ainc-inac.gc.ca/ncp.

Introduction

The Northern Contaminants Program (NCP) was established in 1991 in response to concerns about human exposure to elevated levels of contaminants in fish and wildlife species that are important to the traditional diets of northern Aboriginal peoples. Early studies indicated that there was a wide spectrum of substances – persistent organic pollutants, heavy metals, and radionuclides – many of which had no Arctic or Canadian sources, but which were, nevertheless, reaching unexpectedly high levels in the Arctic ecosystem. The Program's key objective is *to reduce and, where possible, eliminate contaminants in northern traditional/country foods while providing information that assists informed decision making by individuals and communities in their food use*.

Under the first phase of the NCP (NCP-I), research was focussed on gathering the data required to determine the levels, geographic extent, and source of contaminants in the northern atmosphere, environment and its people, and the probable duration of the problem. The data enabled us to understand the spatial patterns and temporal trends of contaminants in the North, and confirmed our suspicions that the major sources of contaminants were other countries. The data, which included information on the benefits from continued consumption of traditional/country foods, was also used to carry out assessments of human health risks resulting from contaminants in those foods. Results generated through NCP-I are synthesized in the *Canadian Arctic Contaminants Assessment Report*.

Extensive consultations were conducted in 1997-1998 to find the common elements between the concerns and priorities of northern communities and the scientific needs identified as critical for addressing the issue of contamination in Canada's North. As a result, priorities for current and future research are based on an understanding of the species that are most relevant for human exposure to contaminants in the North, and geographic locations and populations that are most at risk.

In 1998-1999, the NCP began its second phase (NCP-II), which continued until 2002-2003. Results of this phase are synthesized in the *Canadian Arctic Contaminants Assessment Report II (CACAR II)*. NCP-II supported research designed to answer questions about the impacts and risks to human health that may result from current levels of contamination in key Arctic food species. To ensure a balanced assessment of the risks, an emphasis is placed on characterizing and quantifying the benefits associated with traditional diets. Communications activities are also emphasized and supported under NCP-II. Under the leadership of the northern Aboriginal organizations, the dialogue between northerners and the scientific community, which was initiated in NCP-I, continued to build awareness and an understanding of contaminants issues, and helped to support the ability to deal with specific contaminant issues at the local level.

In addition, the NCP effort to achieve international controls of contaminants remained strong in NCP-II. The legally binding POPs protocol, under the United Nations Economic Commission for Europe (UN ECE) Convention on Long-range Transboundary Air Pollution, has been successfully negotiated and was signed by 34 countries (including Canada) at the UN ECE Ministerial Conference in A?rhus, Denmark in June 1998. Canada ratified this agreement in December 1998. Negotiations for a legally binding global instrument on POPs under the United Nations Environment Programme have

now also been completed with the signing of the POPs Convention in Stockholm, Sweden, May 23, 2001. The Convention has been signed by more than 100 countries; Canada has signed and ratified the Convention. Cooperative actions under the Arctic Council, including the circumpolar Arctic Monitoring and Assessment Programme (AMAP) and the Arctic Council Action Plan (formally launched in October 2000), are continuing. NCP continues to generate the data that allows Canada to play a leading role in these initiatives.

The NCP is directed by a management committee that is chaired by the Department of Indian Affairs and Northern Development, and which includes representatives from four northern Aboriginal organizations (Council of Yukon First Nations, Dene Nation, Inuit Tapiriit Kanatami, and Inuit Circumpolar Conference), the Yukon, Northwest Territories and Nunavut Territorial Governments, Nunavik, and four federal departments (Environment, Fisheries and Oceans, Health, and Indian Affairs and Northern Development). The management committee is responsible for establishing NCP policy and research priorities and for final decisions on the allocation of funds. Three territorial contaminants committees in the Yukon, Northwest Territories and Nunavut (established in May 2000), and a regional contaminants committee in Nunavik support this national committee. Funding for the NCP's $4.4 million annual research budget comes from INAC and participating federal departments.

The NCP Operational Management Guide, available on the NCP website (www.ainc-inac.gc.ca/ncp), provides a summary of the management structures and review processes used to effectively implement the NCP. The Guide explains the overall management structures currently used, the proposal review process and outlines a protocol to be used to publicly disseminate health and harvest information generated by the NCP. Background information on all NCP committees and review teams is also provided.

In 1998, the NCP Management Committee redesigned the NCP-Phase II for application under the 1999-2000 funding year. The two main initiatives undertaken were: 1) the development of blueprints that represent the long-term vision and strategic direction for NCP-II; and 2) the implementation of a more open and transparent proposal review process. This new management structure is designed to ensure that the NCP remains scientifically defensible and socioculturally aware, while at the same time, achieving real progress in terms of the Program's broad policy objectives.

Blueprints were developed for each of the three main NCP subprograms: i) Human Health, ii) Monitoring the Health of Arctic People and Ecosystems and the Effectiveness of International Controls, and iii) Education and Communications. The blueprints are used to provide the necessary guidance to project proponents for the development of proposals as well as to peer reviewers, review teams and the NCP Management Committee for evaluating proposals. They are evolving documents that are reviewed at least annually.

Under a revamped proposal review process, the NCP Technical Committee was replaced with an external peer review process facilitated by review teams. The review of proposals is a two pronged approach involving a scientific review by external peer reviewers, facilitated by technical review teams, and a socio-cultural review facilitated by the regional and Territorial Contaminants Committees (TCCs). Both sets of recommendations are considered by the Management Committee in making final funding decisions. Proposals submitted under the Education and Communications subprogram are evaluated by a technical review team. All peer reviewers, review teams and TCCs use evaluation criteria and the blueprints to review and rate proposals. Written consent from the appropriate northern community authority or national-level Aboriginal organization is required for all projects involving field work in the North and/or analyses of samples as a condition of approval for funding.

This report provides a summary of the progress to date of research and activities funded by the Northern Contaminants Program in 2005-2006. It is a compilation of reports submitted by project teams, emphasizing the results of research and related activities that took place during the 2005-

2006 fiscal years. The report is divided into chapters that reflect the broad scope of the NCP: Human Health; Monitoring the Health of Arctic People and Ecosystems and the Effectiveness of International Controls (including abiotic monitoring and biotic monitoring), Education and Communications, International Policy, and Program Coordination.

Dietary Exposure of Inuit Communities to the Brominated Flame Retardants (BFRs), Polybrominated Diphenyl Ethers (PBDEs)

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Abstract

As of yet little information is available on the presence of the brominated flame retardants (BFRs), in particular polybrominated diphenyl ethers (PBDEs), in northern regions. One hundred and thirty eight samples of country foods collected from different communities in the Northwest Territories and Nunavut were analysed for PBDE.

Key Messages

- PBDEs were detectable in all samples of country food from Inuit and Inuvialuit communities in the Nunavut and the NWT.
- In general marine mammal samples had higher PBDE concentrations than those found in fish and land mammals.
- The dietary intake of PBDEs among Inuit is estimated at less than one microgram per day and is considered to be very low.

Objectives

The overall objective is to obtain a more comprehensive exposure information and risk assessment parameters for Inuit on persistent organic pollutants (POPs) by the analysis of existing country foods from NWT and Nunavut communities. This would be accomplished by:

i) re-analyzing 120 organic extracts of samples of country foods already collected in between 1997-1999 from NWT and Nunavut, that were previously measured for PCB and other organochlorine pesticides, for the brominated flame retardants (BFRs), polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane(HBCDD);

ii) estimating the intake of these POPs for Nunavut communities in the consumption of country foods by combining the levels in foods with the daily food intake and;

iii) communicating the risk of exposure to this compounds in typical Inuit diet, in comparison to that from other communities in the south of Canada, to the two regional contaminant committees.

Introduction

Brominated flame-retardants (BFRs) are a diverse group of chemicals that are added to or reacted with manufactured products such as polymers, plastics, and textiles to reduce their flammability. Currently, there are more than 75 different aliphatic, cyclic-aliphatic and aromatic compounds used as brominated flame-retardants. On the other hand, only a handful of these chemicals are being used in relatively high volumes. Tetrabromo bis phenol A (TBBPA), with an annual market demand of about 120,000 mT, has the highest consumption among BFRs, followed by polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) with an annual market demand of 67,000 mT and 17,000 mT, respectively (BSEF 2003).

PBDEs, like the PCBs, have 209 congeners, are active in the gaseous state and are used to increase the flame resistance of textiles, electronics and polyurethane foam (PUF) (WHO 1994). While the benefits of flame retardants are obvious, their impact on the environment and human health cannot be ignored. Persistent organic pollutants (POPs) are presently being targeted globally for environmental regulation because of its characteristics of accumulation, persistence, toxicity, and potential for long-range movement, which is important for northern regions. PBDE share similar physical and chemical properties to other organochlorine POPs, are being considered for inclusion in this group of compounds and have recently been shown to occur in both abiotic and biotic environment in significant amounts in North America and Europe (Alaee et al. 2003a, Luross et al. 2002, Tomy et al. 2003, Hale et al. 2003, Law et al. 2003). PBDEs have been observed in the Arctic. A recent study showed that PBDEs levels in human tissue from North America on average contains 20 times higher levels of PBDEs than samples collected in Europe (Hites 2004).

It has been established that more than 90% of exposure to POPs occurs through food intake with little or none from air or water. Determination of the dietary exposure to BFRs in Inuit communities therefore, has major significance (Kuhnlein et al. 2000). However, information on human body burdens (uptake) or consumable foods (human intake) is limited. This information is essential for risk assessment, and there are no data for PBDEs in northern country foods from any source.

Activities for 2005/2006

One hundred and thirty eight archived organic extracts from CINE were analysed using GCHRMS methods for PBDEs (Alaee et al. 2001). Briefly, PBDE congeners were analysed using isotope dilution technique with HRGC/HRMS in EI mode with a minimum of 41native, and five ^{13}C labeled PBDE congeners. The laboratory has participated and will continue to participate in international round robin QA/QC programs on PBDEs. The analytical data were merged with the dietary information already available using SAS to estimate intake of these compounds in a typical Inuit diet from different regions.

Results and Discussion

PBDEs were detectable in all samples (Table 1). Highest concentration was found in a Arctic cisco egg sample (36.36 ng/g). In general, marine mammal samples had higher concentrations than those found in fish and land mammals. Estimate intake from different species of animals are presented in Table 2. All food parts and preparation methods of the tissue of the same species are combined. Intake were grouped into different gender (Table 2 a and b) and into 5 different regions. Mean intake from each species was calculated based on participants who reported consuming traditional food as well among all participants (as a proxy for the population average intake). Male had higher intake than female as male consumed more food in general. Among the 5 regions, Baffin had the highest intake as participants in Baffin consumed the highest amount of traditional food. Even though caribou had low concentrations of PBDE (most parts contained less than 10 ng/g), it is the major contributor to PBDE in all 5 regions. In Labrador, caribou contributes almost 90% of PDBE intake. Baffin had the highest PBDE intake at around 1ug per day. The top three sources are caribou, nawhal and ringed seals.

Conclusion

This is the most comprehensive set of data on PBDE in traditional food samples. PBDE were found in all traditional food items. Higher concentrations were found in marine mammals. Intakes of PBDE were generally low, i.e. below 1 ug per day. The major source of PBDE is caribou. We will discuss the results with ITK and the two Regional Contaminant Committees.

Table 1. Concentrations of PBDE measured in Inuit traditional food samples

Food	Part	Preparation	PBDEs ng/g wet wt
Narwhal	Muktuk	Raw	3.49
Narwhal	Muktuk, fat	Raw	5.66
Beluga	Meat	Dried	2.52
Beluga	Blubber	Raw	22.35
Beluga	Muktuk, skin	Raw	3.34
Walrus	Blubber	Aged, raw	3.38
Clams	Whole, no shell	Raw	1.53
Seal, Bearded	Meat	Boiled	1.76
Seal, Ringed	Meat	Raw	1.49
Seal,Ringed	Liver	Raw	1.47
Seal, Ringed	Brain	Raw	0.67
Burbot	Liver	Raw (to cook)	10.05
Burbot	Liver	Raw	26.48
Burbot	Eggs	Raw	4.78
Burbot	Eggs	Raw	3.36
Cisco, Arctic	Eggs	Frozen - raw	36.26
Beluga	Meat-dried	Air-dried /smoked	7.85
Beluga	Meat-dried	Air-dried /smoked	1.55
Beluga	Muktuk, fat	Hung/boiled	7.49
Beluga	Muktuk, skin	Hung/boiled	2.23
Beluga	Oil -fermented from blubber	Cut/smoked/ skimmed	9.18
Beluga	Oil -fermented from blubber	Cut/smoked/ skimmed	20.39
Cisco, Arctic	Eggs	Frozen	0.96
Whitefish, Broad	Flesh	Raw	2.85
Whitefish, Broad	Flesh	Raw	1.74
Whitefish, Broad	Flesh	Boiled	2.10
Whitefish, Broad	Flesh	Boiled	2.64
Char, Artic	Whole	Raw	3.05
Whitefish	Whole	Raw	4.25
Walrus	Meat, fat	Raw	1.66
Walrus	Meat (fat removed)	Raw	2.15
Walrus	Kauk (mattak)	Raw	10.47
Goose, Snow	Heart	Boiled	1.69
Bear, Polar	Leg	Frozen	1.46
Walrus	Blubber	Raw	3.50
Seal, Ringed	Meat	Raw	1.77
Seal, Ringed	Meat	Raw	0.94
Narwhal	Blubber	Raw	24.44
Murr, Thick billed	Meat	Boiled	1.54
Seal, Harp	Meat	Boiled	0.97
Seal, Bearded	Meat	Boiled	1.61
Walrus	Meat	Aged, raw	1.06
Grouse,spruce	Whole	Boiled	0.46
Duck, Eider	Whole	Raw	0.60
Char	Whole, no organs	Raw	1.32
Seal, Ringed	Brain	Boiled	0.82
Duck,Black	Meat	Boiled	1.00
Trout	Whole	Raw	3.24
Grouse,spruce	Whole	Raw	2.97

Table 1. Continued

Table 1. Concentrations of PBDE measured in Inuit traditional food samples

Food	Part	Preparation	PBDEs ng/g wet wt
Char, Artic	No precision	Raw	2.51
Goose	Meat	Raw	0.65
Duck, Eider	Whole	Boiled	0.99
Trout, Lake	Whole	Raw	1.14
Char	Whole fish	Raw	0.69
Char, Arctic	Liver	Raw	1.09
Muskox	Bone marrow	Raw	2.57
Muskox	Ribs	Raw	0.77
Caribou	Liver	Raw	0.66
Seal, Ringed	Meat	Raw	0.71
Seal, Ringed	Blubber	Raw	5.33
Seal, Ringed	Blubber	Raw	3.53
Muskox	Meat, shoulder	Raw	0.95
Seal, Ringed	Meat	Raw	0.83
Beluga	Fat (blubber)	Liquid/raw	15.29
Narwhal	Muktuk	Raw	1.01
Narwhal	Aged oil	Aged	5.50
Beluga	Muktuk, skin	Raw	2.11
Beluga	Muktuk, skin	Raw	2.16
Narwhal	Muktuk	Raw	1.66
Narwhal	Muktuk, fat	Raw	14.83
Narwhal	Muktuk, skin	Raw	1.53
Narwhal	Muktuk, skin+fat	Raw	1.43
Narwhal	Muktuk, skin	Raw	1.01
Seal, Ringed	Oil, not aged	Raw	3.84
Seal, Ringed	Brain	Raw	0.60
Seal, Ringed	Brain	Raw	2.01
Seal, Ringed	Brain	Raw	2.91
Beluga	Muktuk, skin	Raw	2.45
Muskox	Fat	Raw	9.70
Muskox	Fat	Raw	25.93
Loche	Eggs	Raw	2.76
Loche	Eggs	Raw	6.25
Loche	Liver	Raw	20.15
Beluga	Muktuk, fat	Raw	29.99
Beluga	Muktuk, skin	Raw	6.03
Beluga	Muktuk, fat	Raw	18.99
Beluga	Muktuk, skin	Raw	16.40
Beluga	Muktuk, fat	Raw	26.04
Beluga	Muktuk, skin	Raw	9.17
Seal, Ringed	Oil	Raw	5.64
Narwhal	Fat	Raw	16.45
Narwhal	Skin	Raw	4.36
Narwhal	Oil	Raw	4.90
Beluga	Muktuk, fat	Frozen	11.46
Beluga	Muktuk, skin	Frozen	2.59
Beluga	Muktuk, fat	Frozen	13.78
Beluga	Muktuk, skin	Frozen	3.41
Narwhal	Muktuk, skin	Frozen	16.23

Table 1. Continued

Table 1. Concentrations of PBDE measured in Inuit traditional food samples

Food	Part	Preparation	PBDEs ng/g wet wt
Narwhal	Muktuk, skin	Frozen	6.37
Caribou	Liver, (pool)	Baked	4.71
Caribou	Bone marrow	Raw	3.03
Walrus	Flipper	Aged	3.32
Walrus	Flesh, ribs	Raw	3.59
Caribou	Bone marrow	Aged	6.87
Caribou	Bone marrow	Aged	8.00
Walrus	Flesh	Aged	1.17
Caribou	Bone marrow	Raw	9.53
Lake trout	Flesh	Raw	0.34
Lake trout	Flesh	Raw	0.32
Lake trout	Flesh	Raw	0.36
Caribou	Bone marrow	Raw	11.37
Lake trout	Flesh	Raw	1.76
Walrus	Flesh	Raw	16.45
Polar bear	Flesh, ribs	Raw	0.28
Seal	Flesh	Raw	0.63
Caribou	Flesh, chest	Raw	0.44
Arctic char	Flesh	Raw	2.94
Arctic char	Flesh	Raw	2.11
Arctic char	Flesh	Raw	1.68
Arctic char	Flesh	Raw	0.37
Arctic char	Flesh	Raw	0.19
Walrus	Liver	Raw	0.20
Walrus	Blubber	Raw	3.67
Caribou	Flesh	Raw	6.22
Caribou	Flesh	Raw	0.48
Caribou	Flesh	Raw	0.17
Caribou	Flesh	Raw	1.99
Ringed seal	Liver	Raw	0.48
Ringed seal	Liver	Raw	0.18
Ringed seal	Flesh, ribs	Raw	11.47
Ringed seal	Flesh, spine	Raw	0.35
Ringed seal	Flesh, ribs	Raw	3.18
Walrus	Blubber	Raw	13.10
Walrus	Liver	Raw	0.34
Walrus	Liver	Raw	2.17
Walrus	Blubber	Raw	1.53
Walrus	Liver	Raw	0.21
Walrus	Blubber	Raw	2.94

Table 2a. PBDE intake of Inuit participants- in relation to total consumption – FEMALES

Region [total n indiv.[a] / total n TFC[b]]	Species	N	% of PBDE[c] out of total PBDE consumption	PBDE intake averaged over total n indiv. (ng/day)	PBDE intake averaged over total n TFC (ng/day)
Inuvialuit[d]	Bear, polar	1	0.3	1.67	2.80
[176 / 105]	Beluga	9	4.4	28.3	47.5
	Caribou	77	50.0	325	544
	Char, arctic	11	5.8	37.5	62.8
	Cisco/ herring	6	28.0	181	304
	Duck	3	1.4	8.89	14.9
	Goose	1	0.01	0.05	0.09
	Muskox	10	3.1	20.2	33.9
	Seal, ringed	2	2.6	0.01	0.02
	Trout, lake	2	0.4	16.8	28.2
	Whitefish	4	4.1	2.85	4.77
Kitikmeot[d]	Bear, polar	1	0.3	1.84	3.27
[160 / 90]	Beluga	1	5.3	30.9	55.0
	Caribou	63	66.5	391	695
	Char, arctic	20	13.5	79.2	141
	Duck	3	1.7	9.77	17.4
	Muskox	10	3.7	21.9	39.0
	Seal, ringed	3	5.0	29.4	52.3
	Trout, lake	2	1.3	7.84	13.9
	Whitefish	2	2.7	15.7	28.0
Kivalliq	Beluga	7	10.8	81.6	138
[172 / 102]	Caribou	88	71.3	539	909
	Char, arctic	20	11.9	89.7	151
	Seal, bearded	2	1.5	11.0	18.6
	Seal, ringed	2	2.7	20.3	34.3
	Trout, lake	4	1.2	9.13	15.4
	Walrus	3	0.6	4.82	8.12
Baffin	Bear, polar	9	1.4	15.8	23.4
[246 / 166]	Beluga	9	8.2	89.5	133
	Caribou	95	27.2	297	440
	Char, arctic	41	9.7	106	157
	Cisco/ herring	1	1.6	17	25.2
	Clams	2	0.4	4.60	6.81
	Duck	1	0.04	0.46	0.68
	Narwhal	22	24.0	262	388
	Seal, bearded	4	0.5	5.10	7.55
	Seal, ringed	48	17.6	192	285
	Walrus	15	9.4	103	153
Labrador	Caribou	67	83.1	263	477
[196 / 108]	Char, arctic	3	4.3	13.6	24.7
	Duck	3	0.9	2.87	5.21
	Spruce grouse	11	1.8	5.65	10.3
	Seal, ringed	3	4.9	15.5	28.1
	Trout, lake	8	5.0	15.7	28.5

[a]Total number of females participants within the corresponding region.
[b]Total number of females participants with traditional food consumption within the corresponding region.
[c]Foods that did not have PBDE value were substituted by other foods within the same species. When the latter were unavailable, PBDE value was assumed zero (refer to table 1).
[d]One community (Holman) is part of the 2 regions (Inuvialuit and Kitikmeot).

Table 2b. Mean daily traditional food and PBDE intake of Inuit participants- in relation to total consumption – MALES

Region [total n indiv.[a] / total n TFC[b]]	Species (part)	N	% of PBDE[c] out of total PBDE consumption	PBDE intake averaged over total n indiv. (ng/day)	PBDE intake averaged over total n TFC (ng/day)
Inuvialuit[d]	Bear, polar	2	0.8	5.59	8.95
[144 / 90]	Beluga	9	4.6	31.1	49.8
	Caribou	59	57	388	621
	Char, arctic	9	5.1	34.8	55.6
	Cisco/ herring	4	22.4	152	244
	Duck	4	1.3	8.66	13.9
	Muskox	12	3.9	26.5	42.3
	Narwhal	1	0.3	2.05	3.28
	Seal, bearded	2	0.1	0.70	1.12
	Seal, ringed	3	1.6	10.9	17.4
	Trout, lake	1	0.3	2.23	3.58
	Whitefish	3	2.6	17.6	28.1
Kitikmeot[d]	Bear, polar	2	1.2	6.83	11.0
[118 / 73]	Beluga	1	7.3	41.9	67.8
	Caribou	44	62.1	355	574
	Char, arctic	15	16.4	93.8	152
	Duck	3	1.5	8.60	13.9
	Muskox	12	5.6	32.3	52.2
	Seal, ringed	3	2.3	13.3	21.5
	Trout, lake	2	1.1	6.46	10.4
	Whitefish	3	2.4	13.8	22.3
Kivalliq	Beluga	12	21.0	290	442
[122 / 80]	Caribou	69	56.9	786	1199
	Char, arctic	24	12.2	169	258
	Seal, bearded	3	2.6	35.8	54.6
	Seal, ringed	2	1.7	23.3	35.5
	Trout, lake	3	1.1	15.6	23.8
	Walrus	3	4.5	62.0	94.5
Baffin	Bear, polar	5	1.6	16.9	26.3
[212 / 136]	Beluga	2	1.8	19.5	30.4
	Caribou	79	35.9	385	600
	Char, arctic	40	11.6	125	195
	Duck	2	0.2	2.38	3.72
	Muskox	1	0.01	0.09	0.15
	Narwhal	9	17.5	188	293
	Seal, bearded	4	1.4	15.1	23.5
	Seal, ringed	44	21.2	228	355
	Walrus	12	8.7	93.0	145
Labrador	Caribou	61	88.2	384	611
[156 / 98]	Char, arctic	3	3.0	13.0	20.8
	Duck	1	0.3	1.44	2.29
	Spruce grouse	15	3.4	14.8	23.6
	Seal, ringed	2	0.03	0.15	0.23
	Trout, lake	7	5.1	22.1	35.2

[a]Total number of males participants within the corresponding region.
[b]Total number of males participants with traditional food consumption within the corresponding region.
[c]Foods that did not have PBDE value were substituted by other foods within the same species. When the latter was unavailable , PBDE value was assumed zero (refer to table 1).
[d]One community (Holman) is part of the 2 regions (Inuvialuit and Kitikmeot).

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Monitoring Temporal Trends of Human Environmental Contaminants in the NWT: Year 3. MOM'S (Monitoring our Mothers-Study) Environmental Health Trends-Inuvik Region

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Abstract

In May 2003 it was decided to conduct a feasibility study to establish **trends** for human environmental contaminants in the NWT and Nunavut. The new study was approved and began in 2004 as a follow up to the Human Environmental Contaminants Exposure study that was conducted between 1995 and 2001. This baseline study involved the collection of maternal blood samples and information regarding lifestyle and dietary information from pregnant women in the Inuvik region of the NWT and Baffin region of Nunavut. **Consultation** was a key component in this project that began with the initial feasibility study (Year 1), and consultation activities continue as a priority to include meetings with and presentations to: territorial health departments and regional health staff, regional health authorities, territorial environmental contaminants and health committees, regional hospital staff, regional and national aboriginal organizations, including the local Inuvialuit Regional Corporation and the Gwitch'n Tribal Council, communities and representatives of other territorial and federal governments.

Years 2 and 3 of the program included participant recruitment, training of the appointed project coordinator, local health staff and community representatives, collection of hair and blood samples and lifestyle information, and ongoing communication with participants, stakeholders, and team members and the formation of a local informal working group with key health staff and aboriginal partner participation.

Key Messages

- A coordinator was hired for the Mothers Blood Monitoring Program in September 2004
- Training and recruitment began in November 2004 and sampling began in January 2005
- Interest and support for the study continued to be expressed by all stakeholders. Key focus has been on Aboriginal participation to prioritize and improve capacity building and communication
- A proposal for Year 3 of the program was submitted to the NCP and funded. A proposal for Year 4 of the project was submitted and approved in 2006.
- The recruitment and sampling process for this fiscal year has achieved half of the desired 100 participants and will continue until the end of summer in 2006.

Objectives

Short Term Objectives:

i) Maximize, improve and build strong communication networks with territorial and regional health departments, communities and priority Aboriginal organizations

ii) Establishing a temporal trend for maternal exposure to select organochlorine and metal contaminants in NWT and Nunavut

iii) Deliver recruitment materials and general NCP and country food messages while conducting sampling and lifestyle surveys that will be compatible and comparable with the baseline-sampling program in the Baffin and Inuvik Regions.

Long-term objectives:

i) Maximize aboriginal participation, improve capacity building and improve communications relating to all aspects of this project and NCP key messages

ii) Offer a variety of information to Women of childbearing age that offers wise lifestyle choices and enable informed decisions relating to human health and the environment especially as these factors relate to traditional/county food choices

iii) Evaluate temporal trends of maternal exposure to selected organochlorines and metal contaminants in the NWT and Nunavut using blood and hair as biomarkers

iv) Improve our understanding of and describe relationships between contaminant exposure and frequency of consumption of traditional/country foods and select lifestyle factors

v) Contribute to other international blood monitoring programs and to Canada's commitments to Global Monitoring Plans under the Stockholm Convention

vi) Collaborate with other NCP researchers on both study design and sample collection.

Introduction

In May 2003 the Human Health Steering Committee under the Northern Contaminants Program (NCP) met in Calgary and discussed the need to conduct a feasibility study to establish temporal trend monitoring programs of human environmental contaminants in the NWT and Nunavut. The new study will act as follow up to the Human Environmental Contaminants Exposure Baseline conducted from 1995-2001. The main purpose will be to establish a time trend of human exposure to specific environment contaminants through the collection and analysis of human blood and hair samples with expectant women. The Inuvik Study will locally be called: MOM'S (Monitoring our Mothers-Study) and the Environmental Health Trends-Inuvik Region - Expectant Mothers Blood Monitoring Program 2004-2007 will collect information on dietary habits and describe relationships between contaminant exposure and frequency of consumption of traditional/country foods and select lifestyle factors.

Also, this second monitoring study was undertaken in response to study results from the Public Health Research Unit at the CHUL/CHUQ Research Centre that showed beneficial effects of traditional/country food consumption on infant development as well as some subtle negative effects related to contaminant exposure. The monitoring program will be valuable in Canada's effort to meet its international obligation to the Persistent Organic Pollutants (POPs) and Heavy Metals Protocols of the United Nations Economic Commission for Europe (UN/ECE) Long Range Transboundary Air

Pollution (LRTAP) Convention. The program will also contribute data to the Global Monitoring Plan created under the Stockholm Convention that includes human blood as a biomarker.

The Baffin and Inuvik regions were selected as target regions for sample collection. The Baffin region was a priority as it had the highest maternal and cord blood levels of most contaminants in the NWT and Nunavut environmental contaminants baseline study. In contrast, the Inuvik region had the lowest levels of most contaminants in Inuit maternal samples, and overall was in the middle to low range of exposure levels. The Inuvik region also had the highest recruitment rates and the most detailed dietary study of all regions. Data collected from the Inuvik region will allow comparisons between ethnic groups, as it is home to Dene, Metis, Inuvialuit and Caucasian women.

Proposals for the second and third year of the study were submitted to the NCP and approved. Unfortunately delays occurred with staffing and administration issues, thus day to day work on the project was delayed several months, in each of these years. Barbara Armstrong was originally hired as the project coordinator, working under the Inuvik Health and Social Services Authority in September 2004. Administration of the project was moved to the Stanton Region Health Authority in November 2005. Training sessions began and recruitment started in November and sampling began in January 2005. Expectant mothers are now being interviewed around the time of their delivery to assess diet and lifestyle during pregnancy, and are asked to sign a consent form agreeing to provide blood and hair samples for the study. At the time of this report, fifty percent of the recruitment goal of one hundred participants had been achieved. Active recruitment is anticipated to continue until the end of the summer in 2006, final blood and hair samples collected until some time later and then analysis of the final sample results should be returned by the end of the year. These activities and dates are similar to the target achievements of the 'sister' project in the Baffin Region.

Activities in 2005

Years two and three of the study focused on consultations, training, recruitment, and the sampling process with ongoing meetings with a variety of stakeholders to discuss project planning, protocols and communications. The 2005 monthly activities are summarized in the list below:

January-June

- A proposal for Year 3 of the study was prepared and submitted to the NCP
- NCP final year reporting, IRHSSA budgeting, expense reports and billing reconciliation
- Responded to and amended the year three proposal, from the NCP Management Committee
- The data base was set up by Karen Tofflemire in Calgary Alberta
- Inuvik Healthy Babies presentations and participation in various monthly activities
- Meetings with local informal working group set up with Aboriginal representatives from IRC- Alice Thrasher, and GTC- Denise Kurszewski. The Environmental Health Officer at the Inuvik Regional Hospital, Christopher Beverage, Elaine Bright CPNP Regional Coordinator /Nutritionist and Lorraine Walton Manager, Regional Health Promotions.
- Began community visits, starting with Fort McPherson NWT
    - Attended Pre Natal Cooking classes with the CPNP workers
    - Attended community health fair and offered an information table with MOM'S, NCP and Health Country Food Messages, information
    - Started recruitment for upcoming births
- Attended and participated in the NWTECC teleconference calls
- Community visits to Holman, NWT
    - Attended Pre Natal Cooking classes with the CPNP workers
    - Organized a community health fair and offered an information table with MOM'S, NCP and Health Country Food Messages, information
    - Continued recruitment for upcoming births
- Weekly visits at the Inuvik Transient Centre, recruitment and visits with expectant mothers

- 30 lifestyle surveys and blood and hair samples have been completed and sent for analysis

July- March

- Project coordination was changed from the Inuvik Health and Social Services Authority to the Stanton Regional Health Authority
- Participated in several teleconference calls with the Technical Steering Committee to incorporate necessary communications protocols
- Consultation with Erica Myles and Karen Tofflemire in Calgary, AB was ongoing
- Barbara Armstrong attended and presented at the NCP symposium in Victoria, BC with Karen Beddard
- Informal meetings with technical and health steering committee members
- Informal working group reviewed workplan, recruitment, and protocols
- Participated in workshops and gave NCP presentation with Health Workers of the Inuvik Regional Health & Social Services Authority
- Maintained contact with the Gwitch'in Tribal Council (GTC)- (Regional Wellness Coordinators), Denise Kurszewski and Alice Thrasher (IRC) Inuvialuit Regional Corporation as well as the new Health and Environment Coordinator, Catherine Cockney
    - Meetings with Denise, Cathy and Alice- reviewed baseline study and introduction and recruitment booklets
    - Recommendations were offered and then incorporated into the next draft of the recruitment packages, posters design, sample protocols and lifestyle survey
- Attended and participated in the NWTECC teleconference calls and an in-person meeting
- Weekly teleconference calls with Karen Beddard and then Mary Potyrala from the Baffin Region study (Research Coordinators with the Anaana Project: Department of H+SS Government of Nunavut)
- Attended Baffin Region Steering Committee meeting to review communication protocols in Iqaluit, NT
- Community visits to Tuktoyuktuk, Sachs Harbour, Aklavik, NWT
    - Attended Pre Natal classes with the CPNP workers
    - Organized a community health fair and offered an information table with MOM's, NCP and Health Country Food Messages, information
    - Offered NCP presentation and survey training workshop for College Students, while in communities
    - Continued recruitment for upcoming births
- Weekly visits at the Inuvik Transient Centre, recruitment and visits with expectant mothers
- Continued with Doctors and health workers medical rounds and workshop presentations
    - One-day workshop with both Community Health Workers (CHR) and Nurses Orientation Programs. Informal working group participants attended and participated as well as Inuvik Hospital Acute Care workers and lab technicians
    - Separate information sessions for Acute Care workers
    - Offered a half-day introduction to the NCP, long-range contaminants background presentation: Country Foods, Still Healthy Safe and Strong!
    - Reviewed and offered updates on the MOM'S trend study and a lengthy review and training on the lifestyle survey and recruitment, suggested changes incorporated in working documents
- Collaborated on content and design to produce quarterly Canadian Prenatal Program (CPNP) newsletters
- NCP Mid Term reporting
- Class presentations to the Aurora College NRTP (Natural Resource Technology Program)
- Inuvik Healthy Babies presentations and participation in various monthly activities
- Thank you gift bags ordered, organized and recruitment packages re-sent to communities

- Reviewed and updated (ARI) Aurora Research Institute licensing and protocols
- 20 lifestyle surveys and blood and hair samples have been completed and sent for analysis
- Data Base and initial data entry training with Karen Tofflemire in Calgary Alberta

Teleconference calls and communications were ongoing with; Human Health, Technical and Communications Steering Committee members; Jack MacKinnon, Jill Watkins, Jay Van Oostdam, Eric Loring, Erica Myles, Mark Feeley, Constantine Tikhonov and Kami Kandola and Christopher Beverage from the Stanton Territorial Health Authority. As well as; both the hair and blood analysis laboratories, health workers and coordinators, local informal working group, Karen Tofflemire and Jody Walker from the previous baseline study and the NWT Environmental Contaminates Committee.

Results

All Inuvik region and NWT stakeholders that participated in meetings, discussions and conference calls continued to be supportive of the study. In general, stakeholders were aware of the baseline program and understood the importance of conducting the trend monitoring study. There were several significant comments and concerns related to the study design. These included; the inclusion of other Dene communities in the program, administration of project coordinator, the communication of results and building community capacity in the region and concerns regarding increased workloads to the community frontline workers. To date, all these concerns have been incorporated into the overall study design.

Other key issues identified during consultations that have been incorporated and will remain priorities:

- Communication materials should be developed collaboratively and contain clear messages related to public health and healthy pregnancies
- The need for a well developed communication plan for results that included follow up with any women who had blood levels above guidelines
- Visual and oral communication materials for participants and communities are preferred.
- Community visits with the Aboriginal partners will need to be ongoing
- Lifestyle and dietary information collecting during the prenatal interview is of significant interest from a public health perspective.

Table # 1 + 2: MOM'S Communities

Community	Total
Aklavik	7
Inuvik	9
Tuktoyaktuk	10
Holman	8
Paulatuk	2
Tsiigehtchic	3
Fort Good Hope	5
Colville Lake	1
Lutsel K	1
TOTAL	46

Community:	
Number of women	(% of total sample)
Aklavik: 7	(14.6%)
Colville Lake: 1	(2.1%)
Fort Good Hope: 5	(10.4%)
Holman: 8	(16.7%)
Inuvik : 9	(18.8%)
Lutsel K: 1	(2.1%)
Paulatuk: 2	(4.2%)
Tsigehtchic: 3	(6.3)
Tuktoyuktuk: 10	(20.8%)

Table # 3: MOM'S Participants

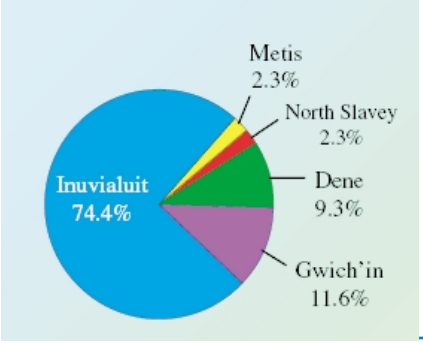
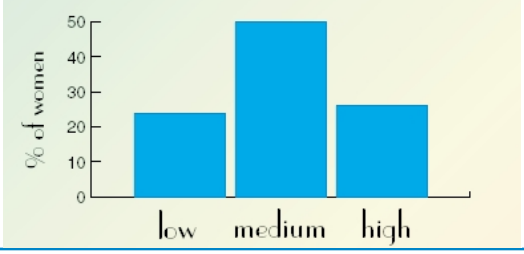


Table # 4: 26.1% of women considered themselves to be high **consumers of country food**, 50.0% medium and 23.9% low



Discussion and Conclusions

The study will continue to involve the recruitment of pregnant women from the Inuvik region of the NWT. Consultations on project planning resulted in the establishment of a recruitment goal of 100 women and to date, over fifty expectant women have participated in the study. Program Coordinators manage the project under the local health authority and undertake all the aspects and responsibilities for the project, including recruitment and sampling. Expectant mothers are interviewed prior to delivery to assess diet and lifestyle during pregnancy, and are asked to sign a consent form agreeing to provide blood and hair samples for the study. Ongoing communications are offered to participants, community frontline workers and health professionals regarding a variety of information related to understanding long-range contaminant health and environment issues, the connections and importance of country/traditional food, and healthy lifestyle choices.

Expected Project Completion Date

March 2007

The Nunavik Health Study: Determination of Dioxin-like Compounds in Plasma Samples from Inuit Adults Using the DR-CALUX Bioassay

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Abstract

Compounds that possess a chemical structure similar to that of dioxin are among the most toxic substances and can elicit a variety of effects in laboratory animals including hormonal disturbances, immune system dysfunction and cancer. Given the paucity of data on exposure of the Canadian Inuit population to these compounds, we set out to analyse plasma samples from 1000 Inuit adults for dioxin-like compounds using a cell-based assay: the Dioxin-Receptor Chemically-Activated Luciferase Expression (DR-CALUX) bioassay, in the course of the Inuit Health in Transition Study that started in Nunavik in 2004. A semi-automated comprehensive extraction multiple fraction (SACEMF) method was elaborated to prepare, from a single plasma sample (~5 mL), the different fractions for GC-MS analyses (project H-11) and the fraction for the DR-CALUX assay. After improving the sensitivity of the DR-CALUX assay and proceeding with the validation of the assay in 2004-2005, we started the analyses of the plasma samples in July 2005. Here we present the data for the first 153 samples that were analysed up to now. The decision to adopt a common extraction scheme with the SACEMF method has resulted in significant delays in the completion of this project.

Key Messages

- A total of 153 plasma samples were analysed for dioxin-like compounds (DLC) using the DR-CALUX assay.
- A moderate correlation was noted between concentrations of PCBs in these samples and concentrations of dioxin-like compounds.
- Analyses of samples will be completed in December 2006.

Objectives

i) To determine the plasma concentrations of dioxin-like compounds in 1000 Inuit adults recruited in the course of the Nunavik Health Study;

ii) To investigate the relations between concentrations of these compounds and those of other persistent organic pollutants (POPs) measured in the course of this cohort study;

iii) To relate DLC plasma levels to dietary habits in the North.

iv) To relate concentrations of dioxin-like compounds to the incidence of chronic diseases documented during the follow up of participants (cohort study).

Introduction

Substances structurally related to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) such as non- and mono-ortho chloro-substituted polychlorinated biphenyls (PCBs) as well as 2,3,7,8-chloro-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), bind the aryl hydrocarbon receptor (AhR) with varying affinities. The ligand-receptor complex triggers the expression of genes that are involved in cell proliferation and differentiation. These compounds referred to as dioxin-like compounds (DLCs), are ubiquitous pollutants that are especially associated with the aquatic food chain. DLCs elicit a number of species-specific, toxic responses in laboratory and wildlife species, including hepatoxicity, body weight loss, thymic atrophy, and impairment of other immune responses, dermal lesions, reproductive toxicity, alterations in vitamin A and thyroid hormone metabolism, teratogenicity, and carcinogenesis (Safe, 1990, 1994).

In humans, a meta-analysis examining cancer risk from dioxin exposure in three occupational cohorts revealed excess risks for all cancers, without any specific cancer predominating (Crump et al., 2003). In specific cohorts, excess risks were observed for reproductive cancers (breast female, endometrium, breast male, testis) but, overall, the pattern is inconsistent (Kogevinas, 2001) and the carcinogenicity of dioxins remains controversial (Cole et al., 2003). Other possible effects linked to DLC exposure in humans include modification of the sex ratio at birth, alterations in thyroid function, and increased risk for diabetes (Kogevinas et al., 2001).

Inuit people residing in the Arctic receive an unusually high dose of organochlorines (OCs) through their traditional diet, which includes large quantities of sea mammal fat. We previously reported results of DLC analysis in 20 pooled plasma samples made of individual samples collected from Inuit adults residing in Nunavik (Arctic Quebec) who participated in a large health survey during the 1990's. The mean total concentration DLCs (expressed in 2,3,7,8-TCDD toxic equivalents) for the 20 pooled samples was 184.2 ng/kg lipids, compared to 26.1 ng/kg lipids for three control pooled plasma samples from Southern Quebec. This DLC body burden in Inuit adults was close to the one that induced adverse health effects in laboratory animals, but this risk remains hypothetical. Only a large scale cohort study conducted throughout the Arctic can generate the results needed to better characterize the risk associated with exposure to DLCs in this population. Such a study has been launched in Nunavik and will involve the participation of 1000 Inuit adults from this territory. In 2006-2007, the cohort study will be expanded to Nunavut (Baffin) and Greenland. Details regarding the cohort study can be found in the synopsis of project H-11 ("Inuit Health in Transition Study: the Nunavik Health Study"; Éric Dewailly, PI).

The conventional method to quantify DLCs in a biological sample involves the determination of the various compounds individually by high-resolution gas-chromatography/high resolution mass-spectrometry (HRGC/HRMS) and knowledge of the relative toxic potencies of individual compounds compared to TCDD, which are expressed as toxic equivalency factors (TEFs). The concentrations of the individual congeners multiplied by their respective TEFs are added up to yield the total TCDD toxic equivalents (TEQ) of the mixture. However, the often small concentrations of individual congeners, the presence of unknown or not routinely-measured AHR agonists, the lack of TEF values for several DLCs, and the possible supra-additive or antagonistic interactions between compounds in a mixture are drawbacks to the TEQ approach. Furthermore, conventional analytical chemistry methods using HRGC/HRMS are expensive and require a large volume of plasma (>10 ml) and extensive sample clean-up. Therefore, in order to study the possible role of DLCs in disease through a large epidemiological study such as the Inuit Health in Transition Study, an alternative to the HRGC/HRMS-based methods is needed.

One such alternative is the DR-CALUX bioassay. In our laboratory, this test is performed using H4IIE.Luc cells (kindly donated by Dr. Abraham Brouwer, Vrije Universiteit, The Netherlands). To produce these cells, a vector containing the luciferase gene under transcriptional control of dioxin-responsive elements was stably transfected into rat (H4IIE) hepatoma cell line that expresses the AHR. Plasma extracts are placed in the cell culture medium and DLCs that are present in the extract diffuse across the cell membrane, bind the AHR and the complex is translocated in the nucleus where it binds dioxin response elements and triggers the expression of luciferase. The response is determined by measuring the intensity of light emitted during

the oxidation of luciferin added to the cell lysate, the rate of this reaction being proportional to the amount of luciferase produced by the cells.

In recent years, several researchers have used this bioassay to measure DLCs in plasma samples in the course of population studies (Ayotte et al., 2005; Covaci et al., 2002; Koppen et al., 2001; Laier et al., 2003; Pauwels et al., 2000, 2001; Van Den Heuvel et al., 2002). In general, moderate to high correlations were observed between results of the DR-CALUX assays and those obtained by HRGC/HRMS. In general, results obtained with the bioassay are somewhat inferior to those obtained by analytical chemistry (HRGC/HRMS), which may indicate antagonistic interactions between compounds in plasma extracts.

Activities in 2005-2006

Analyses of plasma samples from Nunavik Inuit with the DR-CALUX assay have started in July 2005. Because of the large number of samples, the extensive list of compounds to be analysed in the course of the Nunavik Health Study and the limited amount of plasma available, we are using a semi-automated comprehensive extraction multiple fraction (SACEMF) method to prepare the purified extracts to be analysed in this study. Zymark Rapidtrace automated solid phase extraction modules are used to prepare, from a single plasma sample (~5 mL), the different fractions for GC-MS analyses (project H-11) and the fraction needed for the DR-CALUX assay. The various steps of the SACEMF method are shown in Figure 1.

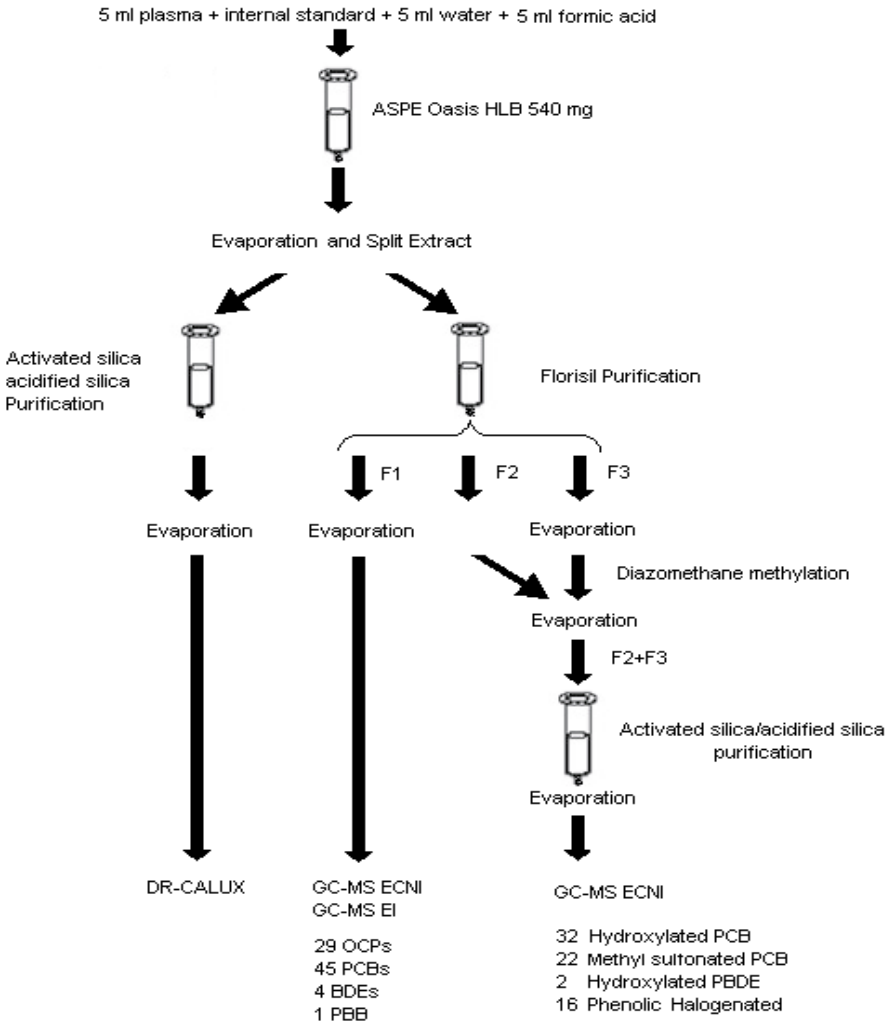

Figure 1. The semi-automated comprehensive extraction multiple fraction (SACEMF) method for POPs analysis in human plasma samples.

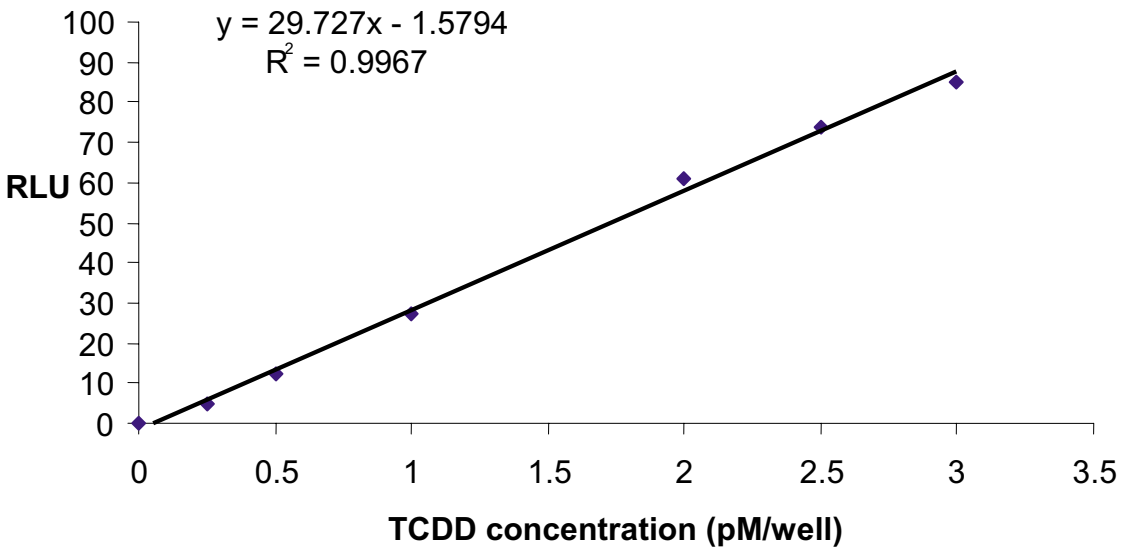

Figure 2. A typical dose-response curve with the DR-CALUX assay.

After extracting the compounds from plasma samples on an Oasis HLB (540 mg; Waters Corp.) solid phase extraction column and eluting the compounds with 10%MeOH/dichloromethane, half of the eluate is used for the DR-CALUX assay. The CALUX fraction is evaporated to dryness and then reconstituted with 5 µl of dimethylsulfoxide before being analysed for DLCs with the DR-CALUX assay. The cells are plated at a density of $8x10^4$ cells/well in 24-well plates. After 24h, the 2,3,7,8-TCDD standards and plasma extracts were added on the cells for 24 hours. The cells are then washed in PBS and lysed in lysis buffer (Promega). The luciferase activity is determined with a luminometer (LMax Molecular Devices).

Results

More work was performed to further improve the precision and the sensitivity of the DR-CALUX. Similarly to Jonas et al. (2004), we are now using a linear curve fit for the TCDD calibration curve. Figure 2 shows a typical linear calibration curve obtained the DR-CALUX assay. The linear relationship is observed up to 3 pM TCDD per well. The limit of detection is now down to 0.25 pM/well, corresponding approximately to 30 pg TEQ/L.

The distribution of values obtained for the 153 plasma samples analysed up to now is shown in Figure 3. The distribution is clearly skewed right, with a mean concentration of 78.6 pg TEQ/L, a median concentration of 53 pg TEQ/L, with individual values ranging from < 30 to 492 pg TEQ/L.

We examined the correlation between plasma DLC concentrations and the concentrations of polychlorinated biphenyl congener 153 (PCB 153), the most abundant PCB congener in plasma samples of participants (Figure 4). The Pearson correlation coefficient calculated using log-transformed values indi-

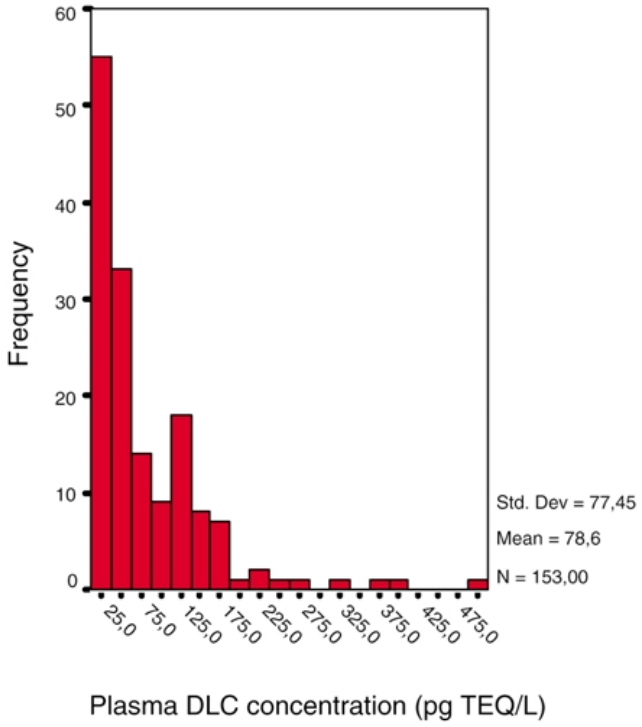

Figure 3. Frequency distribution of plasma DLC concentrations in 153 Inuit adults from Nunavik, 2004.

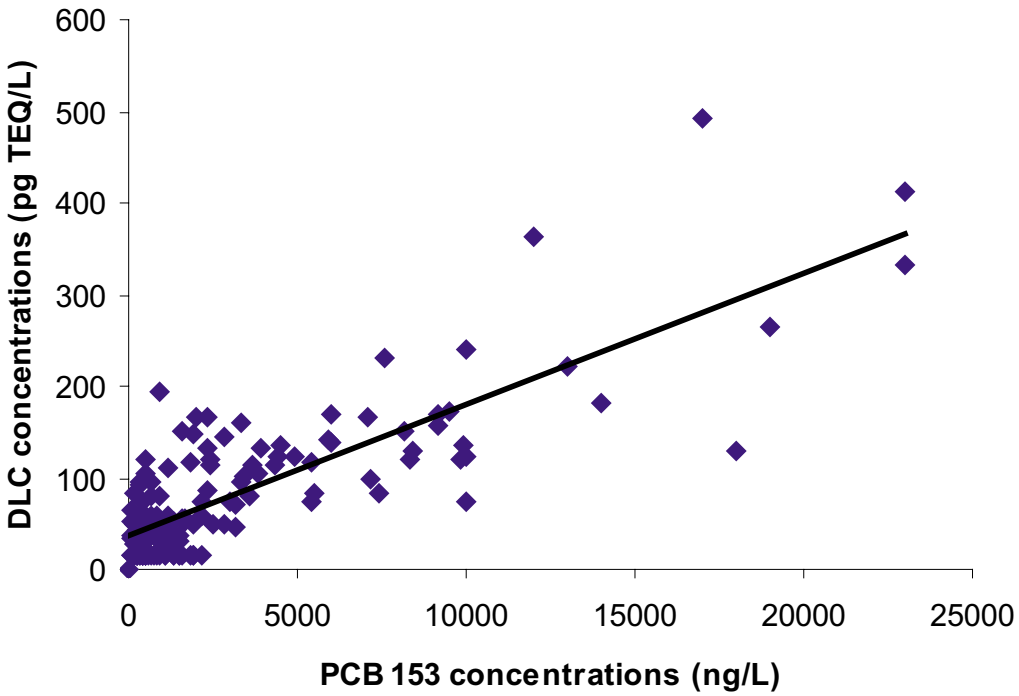

Figure 4. Correlation between plasma concentrations of DLCs determined by the DR-CALUX bioassay and plasma concentrations of PCB 153 determined by HRGC/MS. (Pearson's r = 0.66; p < 0.001; log-transformed values).

cates that a moderate correlation exists between DLCs and PCBs (r = 0.66, p < 0.001).

Discussion and Conclusions

Because of the large number of samples and analytes included in the Nunavik Health Study, a semi-automated solid phase extraction procedure had to be set up and validated. The development of this method delayed DLC analyses with the DR-CALUX. In the meantime, we worked on improving the precision and the sensitivity of the DR-CALUX assay in our laboratory. We have started the analysis of the samples in July 2005. The moderate correlation observed between DLCs and PCBs suggest that PCBs are responsible at least in part for the dioxin-like activities measured in plasma extracts. A more thorough analysis of factors associated with DLCs in plasma samples of participants will be performed once the analyses with the DR-CALUX assay will be completed in December 2006. Since the DR-CALUX bioassay can be conducted with as little as two ml of plasma, at a fraction of the cost of conventional analytical chemistry methods, it can be applied in the course of large scale epidemiological studies such as the Inuit Cohort Study. This will allow the examination of possible associations between exposure to dioxin-like compounds and the incidence chronic diseases in the Inuit population.

Expected Completion Date

March 31, 2007.

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Neurotoxicological, Thyroid and Systemic Effects of *in Utero* and Lactational Exposure to Polybrominated Diphenyl Ethers (PBDE) in Sprague-Dawley Rats

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Abstract

Exposure to polybrominated diphenyl ethers (PBDE) has been increasing in North American populations for the last 20 years. While there is little data on human exposures in Canadian Arctic populations, PBDE have been found in the Arctic environment and wildlife. Because aboriginal Arctic populations rely on wildlife as part of their diet, it is likely that they are also exposed to PBDE and there is concern that levels of it may be increasing faster in the Arctic than elsewhere. Because PBDE have structural similarities to PCB, it is thought that neurobehavioural function, thyroid function and cancer are the most likely consequences of PBDE exposure and that fetuses and infants are the most susceptible populations. This study evaluates the toxicity of DE-71, a commercial mixture of PBDEs, in offspring of rodents exposed during gestation and lactation. Results indicate that PBDE readily entered the brain of offspring and produced higher tissue levels than in mothers. The highest dose tested (30 mg/kg/day) produced in maternal and offspring weight gain but these decreases were transient. In addition, PBDE altereds thyroid hormone levels and CYP-450 enzyme activities in both mothers and offspring; however, offspring were about ten times more sensitive than mothers with 3 mg/kg producing significant alterations. Exposure to 30 mg/kg PBDE decreased motor activity in young rats and mature offspring and this effect appeared to be persistent. Indeed, in mature adults 3.0 and 30 mg/kg decreased motor activity. While biomarkers of brain cholinergic expressions were altered in young and mature animals, there was no indication that responsiveness in old animals to the cholinergic agonist nicotine was altered by perinatal PBDE exposure. There was no indication that learning or memory is affected by PBDE. Similarly, impulsivity didoes not appear to be affected by PBDE. Reactivity measurements indicated that the highest PBDE dose increased reactivity and may have blocked the normal decrease in anxiety levels associated with experiences with novel environments.

Key Messages

- This study evaluated the neurotoxic and systemic effects of gestational and lactational exposure to DE-71 (a commercial mixture of polybrominated diphenyl ethers (PBDE)) in rodents,

- PBDE readily enters the brain of offspring exposed in utero and during lactation, and brain levels are greater than blood levels up to at least weaning at postnatal day (PND) 21.
- Exposure to PBDE at 30 mg/kg produced small but transient alterations in maternal and offspring growth but did not affect mortality rates,
- PBDE decreasesd thyroid hormone levels in mothers and offspring but offspring were about 10 times more sensitive than mothers,
- PBDE altered cytochrome P-450 drug metabolising enzyme levels in mothers and offspring but offspring were about 10 times more sensitive than mothers. Similarly, PBDE altered clinical chemistry in PND 21 offspring but had no effect on mothers,
- PBDE altered motor activity into adulthood, and altered reactivity but did not appear to affect learning, memory or impulsivity

Objectives

Evaluate the effects of perinatal exposure to DE-71 on physical growth and development, thyroid function as well as systemic toxicity,

i) Determine the levels of PBDE in brain and blood in young animals exposed to DE-71 during gestation and lactation and examine the relationship between levels found and specific neurobehavioural, cholinergic, thyroid and systemic effects,

ii) Evaluate the long term consequences of *in utero* and lactational exposure by assessing neurotoxicological effects during early development, in juveniles and in adults to determine the permanency of health effects as well as the occurrence of delayed neurotoxicological health effects.

Introduction

Polybrominated diphenyl ethers (PBDE) are a group of chemicals that have a chemical structure similar to polychlorinated biphenyls (PCBs) and like PCBs, also appear to be persistent in the environment (Schecter, Pavuk et al., 2003). PBDE are found in humans tissues and there is evidence that levels have been increasing in the last 20 years (Schecter et al., 2003; McDonald, 2002; Birnbaum and Staskal, 2004; Hooper and McDonald, 2000; LoPachin, 2004) especially in the Arctic environment, where levels may be increasing at a faster rate than elsewhere (Northern Contaminants Program, 2003). Consistent with this, PBDE have been found in wildlife (Wilson, Ikonen et al., 2004; Utgikar, Chaudhary et al., 2004) that can form a substantial part of traditional country diets. The limited toxicological information available on PBDE indicates that the most likely adverse health effects of exposure are: a) neurobehavioural effects; b) thyroid effects (possibley a contributor to neurobehavioural effects); and c) cancer (McDonald, 2002). A no observable effect level (NOEL) has been established for PBDE (McDonald, 2002).

A small number of studies have shown that PBDE affect neurobehavioural function following perinatal exposure or single exposures to relatively high doses (e.g., 8-10 mg/kg) (Eriksson, Viberg et al., 2002b; Viberg, Fredriksson et al., 2003). The limited data available have been somewhat inconsistent but suggest that, similar to PCBs, PBDE congeners (e.g., PBDE 99) may exhibit congener specific toxicity that may not be reflected by PBDE mixtures (e.g., DE-71). Since the prominent congeners in DE-71 (47, 99, 153) are the ones most frequently found in human tissues (Birnbaum and Staskal, 2004) and in approximately the same proportions, experimental DE-71 exposure may provide a more realistic assessment of the potential neurotoxic and thyroid effects in exposed humans. Moreover, like PCBs, most researchers believe that the most sensitive populations to PBDE exposure are likely to be fetuses and infants (McDonald, 2002).

This study examines the impact of perinatal exposure of rodents to the DE-71 mixture on growth, neurobehavioural development, neuropathology, cholinergic receptor function (assessed both behaviourally and biochemically) and thyroid function. In addition, because there is limited information on systemic toxicity of PBDE after perinatal exposure, we will assess systemic toxicity in these animals. We also measure blood levels of the major PBDE congeners in the exposed mothers and tissue levels (blood and brain) in the exposed offspring. The study design permits us to provide toxicological information on a range of health endpoints and to correlate toxicological effects with tissue exposure levels and to permit comparison with threshold effects levels expected in human populations. The results of this study will be relevant to Arctic populations by developing

toxicological knowledge of exposure to PBDE. Once exposure levels can be characterized in this population, this knowledge can then be employed to determine if adverse health effects are likely and if remediation strategies are required.

Activities in 2005-2006

The animal phase for the main study has been completed. Pregnant rats were dosed with PBDE from gestation day 1 to weaning at postnatal day 21 (PND 21). All reproductive, growth and early developmental measurements have been collected. All tissues have been collected from offspring at PND 4, 11, 14, 21, 50 and 100, 250 and 460 and at PND 21 in dams. Residue analysis has been completed. Analysis of liver metabolizing enzymes from dams and offspring at PND 21, 50, 100, and 250 has been completed. Haematology and clinical chemistry from dams and offspring at PND 21, 50, 100 and 250 and 460 has been completed. Analysis of cholinergic receptors and acetylcholinesterase in brain in offspring at PND 21, 50, and 215 has been completed. Analysis of serum thyroid hormone levels in dams and PND 21 and in offspring at PND 21 and 50 has been completed. Analysis of thyroid morphology in offspring at PND 21 has been completed. Neuropathology analysis for offspring at PND 11 and 21 has been completed. All measurements of neurobehavioural function in offspring have been collected including: activity (PND 16, 55 and 110), nicotine-stimulated activity (PND 450), startle (PND 20 and 90), motor co-ordination (PND 33 and 60), emergence latency (PND 35, 82), learning and memory in Morris Water maze (PND 240 and 260) and delayed spatial alternation (PND 150-200), impulsivity (DRL at PND 245).

Results

Reproduction and Growth: PBDE exposure exerted little impact on reproduction and growth. No dose affected litter size or mortality rates. The highest PBDE doses (30 mg/kg) produced a significant decrease in offspring weight but this did not persist into adulthood and normal body weight was evident by PND 50 and after. Maternal weight was reduced by about 10% during gestation and early lactation but maternal weight had returned to normal values by the end of lactation at PND 21.

Residue Analysis: Residue analysis in offspring indicated that all doses increased PBDE levels in blood (plasma or serum) and brain in offspring at PND 4 (Figure 1)). Tissue levels of PBDE congeners increased 10-fold with a 10-fold increase in maternal dose. Results indicate that blood and brain levels were comparable indicating that PBDE congeners are readily distributed to brain. Serum concentrations were further increased at PND 11 (not shown). At weaning (PND 21, Figure 2), tissue levels were decreased relative to PND 4 likely reflecting reduced exposure by weaning combined with rapid growth. Interestingly, brain levels were higher than serum levels at PND 21 for all PBDE doses indicating that PBDE congeners are readily absorbed into brain tissue at weaning. Note that, although not shown, the same pattern was evident for other PBDE congeners (PBDE 49, 66, 85) although these occurred at lower concentrations. Maternal blood levels at PND 21 were 8-10 times lower than offspring blood levels at PND 21.

Systemic Toxicity: Haematology measures in dams were not affected by any PBDE dose. While PND 21 pups showed reduced red-cell counts (RBC), haemoglobin (Hb), and haematocrit (Hct) relative to mothers, this is not unusual as the pups have not fully developed their bone marrow erythroid maturation. PBDE did not affect haematology in offspring at PND 21 or 50.

PBDE did not affect any measures of clinical chemistry in dams. In offspring at PND 21, there were reduced levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose and uric acid in the 30 mg/kg PBDE dose group. The highest PBDE dose also produced a significant elevations in cholesterol, total protein and albumin levels. The elevation in cholesterol is most likely linked to induction of liver cytochrome P-450 enzymes. The effect of PBDE on clinical chemistry was no longer evident by PND 50 or afterwards.

The highest PBDE dose (30 mg/kg) produced a significant induction of the cytochrome P-450 dependent drug Metabolizing enzymes EROD, BROD, and PROD in dams. When expressed as a percentage of control animals the magnitude of induction was 2944%, 1586% and 1043% for EROD, BROD and PROD, respectively. This is consistent with the increased relative liver weight that was seen in the dams exposed to 30 mg/kg of PBDE. In offspring at PND 21 the two highest doses of PBDE (3.0 and 30 mg/kg) produced significant increases in EROD,

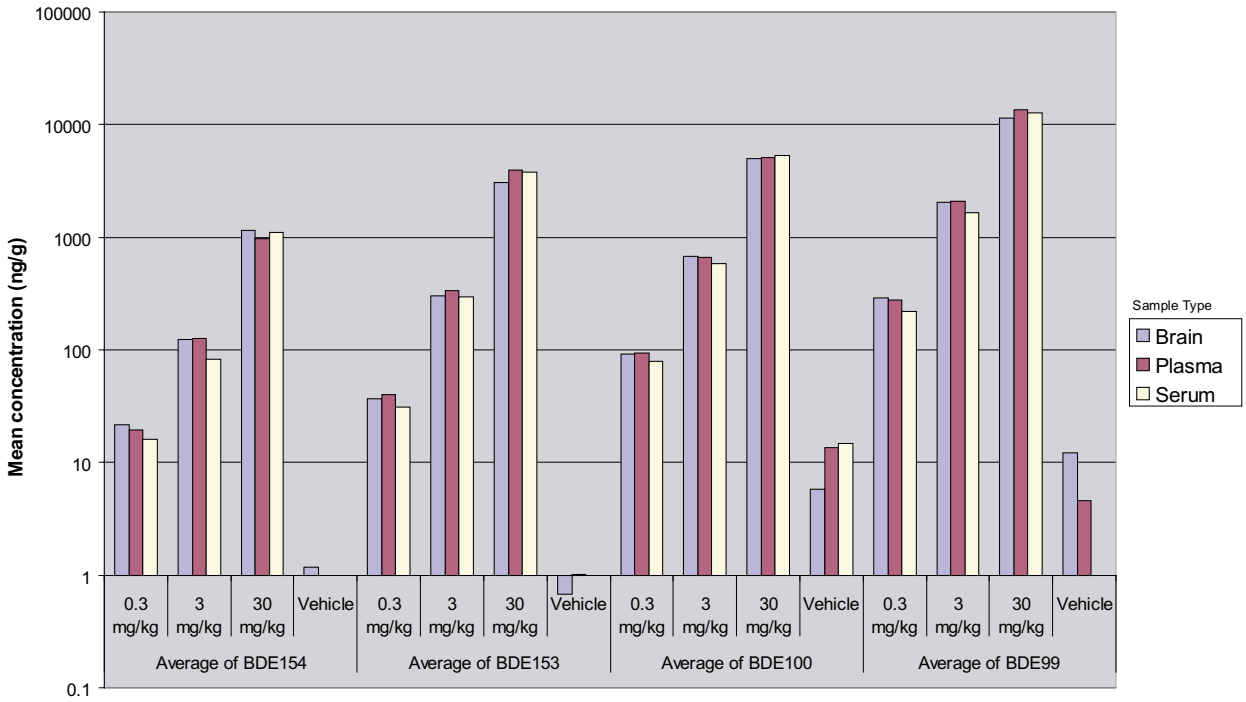

Figure 1. PBDE Concentration at PND 4

BROD and PROD levels, about 2 times the increases over control values than observed in the dams. Further, while 3.0 mg/kg PBDE did not significantly increase EROD, BROD or PROD in dams, it did produce significant increases in 21 day pups. The highest dose of the PBDE also increased liver weight in PND 21 offspring. All clinical measures were normal at 53, 100, and 250 days; however, 30 mg/kg PBDE lowered BROD and PROD levels in males PND 250. While the cause of this has not been determined, BROD and PROD values had returned to normal ion all groups by PND 450.

Thyroid toxicity: Exposure to PBDE also altered thyroid hormone levels. In dams, 30 mg/kg PBDE decreased serum T3 and T4. Similar to cytochrome P-450 enzymes, serum T3 and T4 levels in offspring exhibit a ten-fold greater sensitivity to PBDE than in mothers. At PND 21, levels of serum T3 and T4 were significantly reduced by 3 and 30 mg/kg PBDE. By early adulthood serum T3 and T4 levels has returned to normal in offspring. In addition, serum thyroid stimulating hormone (TSH) levels were significantly elevated in PND 21 offspring exposed to 30 mg/kg PBDE. Finally, 30 mg/kg PBDE altered thyroid morphology (epithelial height) in PND 21 offspring.

Neurobehavioural measures: Perinatal exposure to PBDE altered a number of behavioural measures in young, juvenile and adult offspring. The highest PBDE dose (30 mg/kg) altered days to occurrence of eye opening in females and days to ear opening in females. In both cases, these effects were modest and are not considered to be of toxicological importance. The highest PBDE dose also produced a small decrease in grip strength at PND 12 and 15 but this was not statistically significant.

Motor Activity: Motor activity was measured at PND 16, 55 and 110. The highest PBDE dose decreased motor activity in young (PND 16) and mature (PND 110) but not in juvenile (PND 55) or old rats (PND 450), though the decrease was marginally significant in the youngest animals (Figures 3 and 4). Furthermore, at PND 110, both 3.0 and 30 mg/kg PBDE decreased motor activity suggesting that

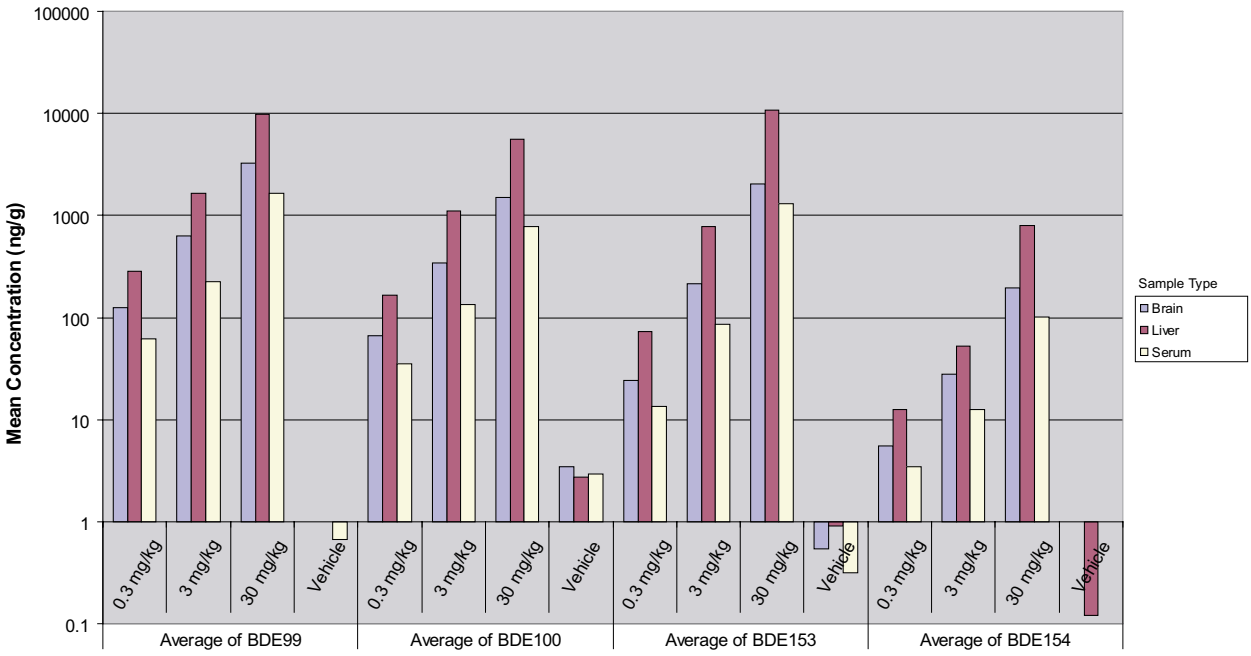

Figure 2. PBDE Concentration at PND 21

delayed effects of perinatal exposure to the intermediate dose may not become evident until adulthood. As expected, at PND 16 there was no temporal pattern suggestive of habituation, while clear patterns of habituation were evident at PND 55 and 100 and 450. We found no indication that exposure to PBDE altered temporal patterns of motor activity at any age. We also evaluated nicotine-stimulated activity in old rats (PND 450) to determine if responsiveness of cholinergic neurochemical stimulation was affected by perinatal exposure to PBDE. While nicotine produced the expected decrease in motor activity and the normal gender difference in activity was Figure 3 and Figure 4 present, no dose of PBDE altered the effect of nicotine on motor activity in old rats.

Motor co-ordination: (beam test) was conducted at PND 33 and PND 60. The two principle measures obtained were latency to fall and distance travelled. PBDE dose did not affect the proportion animals falling. While a larger proportion of males than females fell, this was unrelated to PBDE dose. This likely indicates that even at this age the larger males find this task more challenging than females. Among animals that did not fall, 0.3 mg/kg PBDE increased distance travelled in females but not males. In young adults at PND 60, mature animals were tested on the beam for two trials. Few animals fell off the beam at PND 60. There was a significant increase in distance travelled between trial 1 and 2 as would be expected based on a practice effect. However, PBDE had no impact on either proportion falling or distance travelled. These data suggest that PBDE produces no overt motor impairments in offspring.

Emergence Latency: Emergency latency (or light-dark test) provides a measure of fearfulness or emotional reactivity. This 10 min test was conducted in young juveniles (PND 35) and mature animals (PND 82). Because half the animals tested had previous experience in the activity chambers (i.e., reduced novelty), we also evaluated the role of previous experience with the environment in this test. At PND 35, 0.3 and 3.0 mg/kg PBDE increased latency to emerge relative to vehicle and 30 mg/kg PBDE but only in animals with previous experience in the chambers. Experience alone reduces latency to emerge as would be expected based on reduced

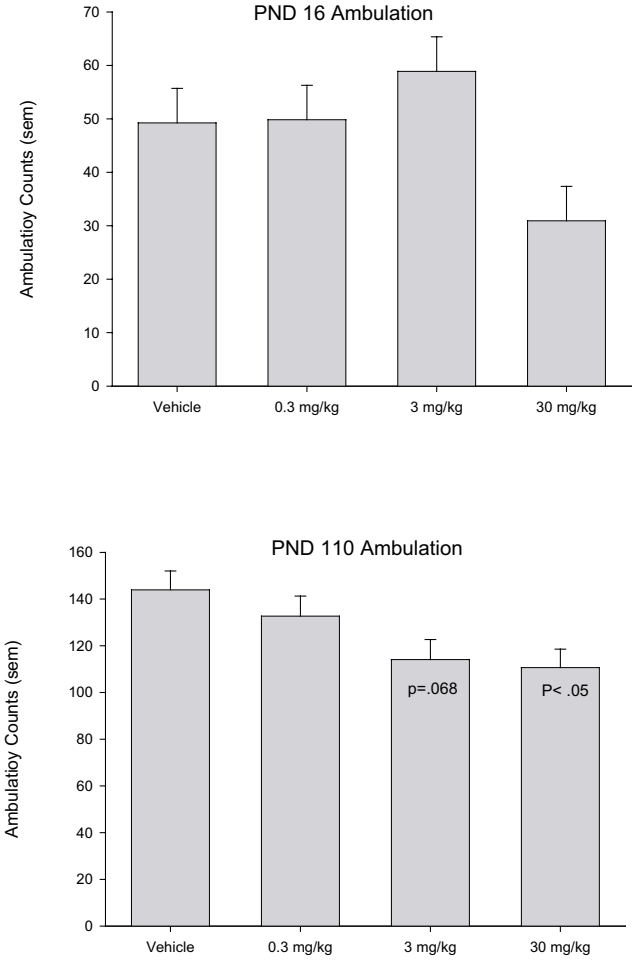

Figure 3. Motor activity, measured as ambulatory counts, in rat pups at PND 16 and 110 under four different PBDE exposure scenarios.

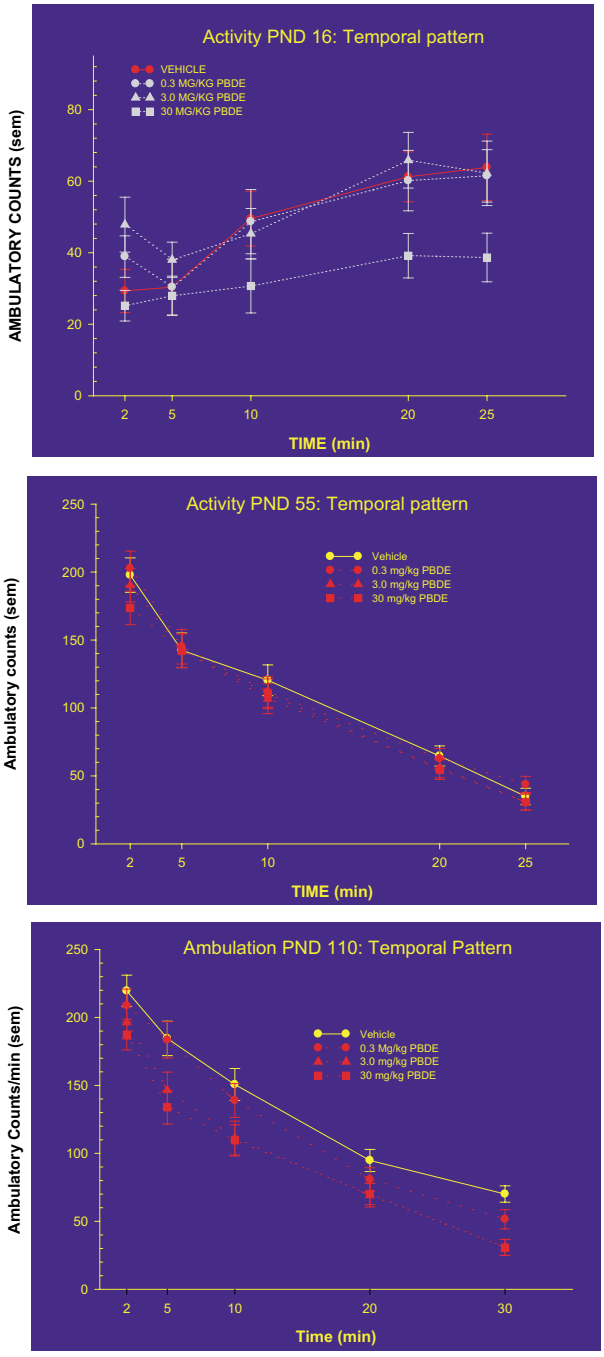

Figure 4. Motor activity measured several times over a 25 minute period, in rat pups at PND 16, 55, and 110 under four different PBDE exposure scenarios.

novelty. The low doses of PBDE appear to block the normal decrease in emergence latency produced by prior exposure to the test environment. This suggests that PBDE either attenuates normal habituation associated with repeated exposure to an environment, or produces a generalized increase in reactivity that is maintained despite prior exposure to the test environment. In mature offspring (PND82) PBDE did not affect emergence latency.

Acoustic Startle: Acoustic startle was conducted at PND 20 and PND 90 to evaluate reactivity, sensorimotor integration (pre-pulse inhibition) and habituation of the startle response. At PND 20, no PBDE dose altered startle responses or prepulse inhibition of startle responses were detected. Normal habituation of the startle response, however was present at PND 20. At PND 90, the highest dose of PBDE (30 mg/kg), increased startle response magnitude but only at the highest startle intensity (Figure 5). PBDE did not affect either prepulse inhibition or habituation of the startle response.

Morris Water Maze: Learning and memory were assessed in the Morris Water Maze in mature offspring (PND 240-260). Animals are trained to locate a submerged platform based on cues outside the

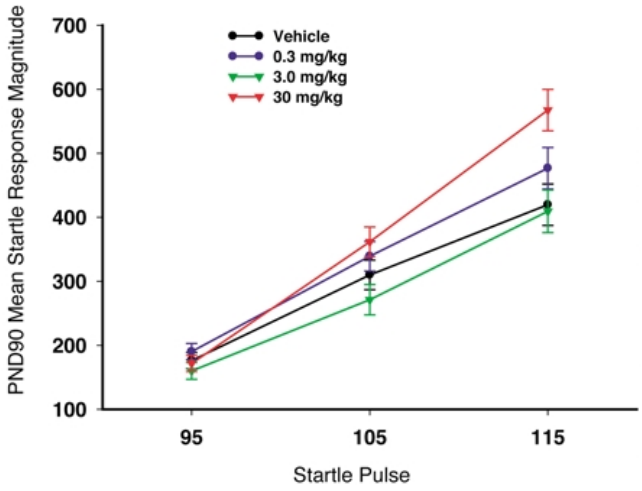

Figure 5. Mean startle response versus startle pulse at PND 90.

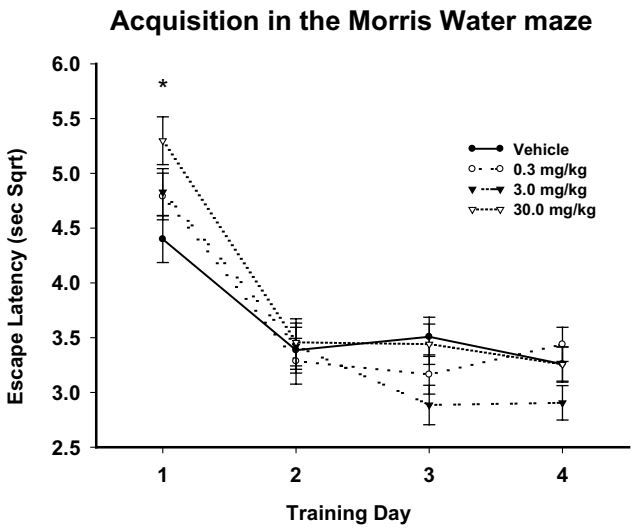

Figure 6. Acquisition in the Morris Water maze

platform. After four days of training, spatial memory is evaluated on the fifth day by measuring the time the animals spend in the former platform location two trials after the platform was removed. Results indicated that all groups learned to locate the escape platform over training days with maximum performance evident after 3 days (12 trials). Though there were minor differences between genders in learning to locate the escape platform PBDE had no effects on learning rates. On probe trials to evaluate PBDE, there was no impact on time spent in the platform area or number of platform crossings on trial 1 suggesting that memory was not affected by any PBDE dose. For vehicle treated animals distance to platform area and duration in platform area were comparable to trial 1. In contrast, all PBDE treated animals increased time in the platform area and decreased mean distance to the platform from trial 1 to 2. While these results provide little evidence for impaired memory, they provide some evidence that the highest PBDE dose may affect the nature of behavioural search strategies compared to vehicle control animals, suggestive of cognitive processing alterations.

Delayed reinforcement of low rate: This test is designed to evaluate the ability of animals to withhold responding during a short delay and provides a measures of impulsivity. Offspring were tested at PND 245. As expected, as the delay interval was increased, the number of premature responses in the delay interval increased. There was no difference between males and females and PBDE has no impact on delay response errors. It should be noted that animals were tested only to delays of a maximum of 7 sec and it is not clear whether PBDE-related impairments would be evident at longer delays.

Neuropathology: Tissues have been collected, sections fixed and stained and slides are currently under evaluation. Pathological evaluations on brains provided little suggestions of any overt pathological changes. Additional detailed, morphometric measurements in PND 21 animals will be conducted.

Cholinergic neurochemistry: Normal development of muscarinic acetylcholine receptors is important for learning, memory and cognitive function. Cholinergic neurons make contact with non-cholinergic neuronal structures suggesting that they modulate the activity of other types of neurons including modulating the release of other neurochemicals such as norepinephrine, dopamine, GABA, and serotonin. Because of it's heavy innervation of hippocampal and midbrain structures, these two areas were initially analysed. Results of cholinergic system biomarker analysis indicate that PBDE differentially affected males and females in immature offspring (PND 21) but not mature offspring (PND 245). At PND 21, 30 mg/kg PBDE increased expression of muscarinic receptors subtypes M2, M3 and M5 and nicotinic receptor subtypes N3, N5, and N7 and acetylcholinesterase in female offspring. In males

Table 1. Biomarker analysis in the midbrain of the immature (PND 21) and mature (PND 245) offspring

	PND 21				PND 245			
	Females		Males		Females		Males	
Biomarker	0.03 mg/kg	30.0 mg/kg	0.03 mg/kg	30.0 mg/kg	3.0 mg/kg	30 mg/kg	3.0 mg/kg	30 mg/kg
M1	nc	1.6	2.3	nc	-2.0	-2.3	nc	-2
M2	nc	2.8	nc	nc	-2.5	-2.5	-2	-2
M3	nc	2.6	nc	nc	-2.4	-2	-2	-2.5
M4	nc	1.2	2.7	nc	-2	-2	nc	-2
M5	nc	4	nc	nc	-3.2	-2.4	-2	-2.5
Ache	nc	4	nc	nc	-1.7	-1.7	-2	-2
nAche2	nc	nc	nc	nc	-2	-2	nc	-2
nAche3	nc	3.0	nc	nc	-2	-2	nc	-2.6
nACh5	nc	3.0	nc	nc	-2.8	-5	-3	-5
nAChe7	nc	2.0	nc	nc	-2.0	-2	nc	-2

Abbreviations: nAChe- nicotinic acetylcholine receptors-, M1-M5- muscarinic acetylcholine receptors (subtypes M1-M5). Ache-acetylcholinesterase. The negative values represent the down-regulation of the gene relative to the vehicle group. The positive numbers represent the up-regulation of the gene relative to the vehicle group.

the lowest PBDE dose (0.03 mg/kg) increases expression of muscarinic receptor subtypes M1 and M4. In mature offspring (PND 245) PBDE down regulated expression of muscarinic and nicotinic receptor subtypes in males and females at the highest PBDE dose. The intermediate PBDE dose produced comparable effects in females but not males. The lowest PBDE dose did not alter any biomarker at PND 245.

Discussion and Conclusions

Results indicate that gestational and lactational exposure to the commercial PBDE mixture, DE-71, produces effects on maternal and offspring growth; however, this occurs only at the highest dose (30 mg/kg/day) and both mothers and offspring appear to recover from the effects of PBDE on growth. Results from systemic toxicity evaluation indicate that liver metabolizing enzymes are activated in both dams and PND 21 offspring. While no dose of PBDE affected clinical chemistry in dams, clinical chemistry was altered in offspring exposed to the highest PBDE dose. Similarly, while only the 30 mg/kg/day doses decreased serum T3 and T4 levels in dams, both the 3.0 and 30 mg/kg/days doses decreased T3 and T4 levels in offspring. Brain biomarker measures in PND 21and 245 offspring indicate that brain cholinergic systems are altered in young and mature offspring but effects are gender-dependent with males and females exhibiting a differential pattern of expression as well as differential sensitivity to PBDE. While learning, memory and impulsivity do not appear to be affected by PBDE, motor activity is decreased in young and mature offspring and the intermediate doses affected activity in adults but not in offspring suggesting delayed effects of perinatal exposure. Motor activity data, however, provided no evidence that habituation was affected. In addition, the highest dose altered reactivity in adults and the two highest doses may have blocked the normal decrease in anxiety or fearfulness associated with novel environments. Taken together the results indicate that systemic toxicity, endocrine, neurochemical and neurobehavioural effects weare present in offspring exposed to PBDE during gestation and lactation.

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Developmental Neurotoxicity of a Persistent Organic Pollutants (POP) Mixture Mimicking the Exposure of Canadian Northern Populations: Correlation of Brain Genomic and Proteomic Data with Thyroid Physiology and Neurobehavioural Outcomes

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Abstract

The Northern Contaminant Mixture, containing the 27 most abundant pollutants found in the serum of Canadian Arctic mothers, was shown to affect rat neurodevelopment (Chu et al., 2003). The effects of the Northern Contaminant Mixture on pup growth, survival, endocrine functions, neurochemistry and neurobehaviour were then compared to those of methyl mercury (MeHg), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) administered separately at the same concentration found in the Northern Contaminant Mixture (Chu et al., 2004, 2005; Desaulniers et al., 2005). This report focuses on cerebellum and hippocampus gene expression profiles resulting from such exposures. A positive control group for mild hypothyroxinemia was added to this study by dosing rats with the goitrogenic agent propylthiouracil. Polybrominated diphenyl ethers (PBDEs), treatment groups were also added, owing to their increasing environmental concentration and structural similarity with PCBs. Global gene expression profiles of hippocampus and cerebellum samples clustered reasonably well according to their treatment group and some differentially expressed genes could be linked to known molecular mechanisms of neurotoxicity. However, significant interactions within Northern Contaminant Mixture prevent accurate and reliable prediction of gene expression based on the list of components present in this mixture. We also observed that some thyroid hormone driven effects may take place in the brain in absence of noticeable perturbation of blood thyroid hormone levels. This study will open avenues for hypothesis driven research to gain insights on molecular mechanisms of toxicity, to develop biomarkers of exposure/effects, and test potential ameliorative approaches to mitigate the effects of environmental contaminants.

Key Messages

- We compared the effects on molecular endpoints of the Northern Contaminant Mixture to the effects of the major components of this mixture administered separately.
- Gene expression profiles of cerebellum and hippocampus samples clustered reasonably well according to their treatment group, but the response of a specific gene following exposure to

the Northern Contaminant Mixture can't be reliably predicted from the list of the mixture's components.

- Despite their failure to affect serum thyroid hormone levels, organochlorine pesticides caused a significant thickening of thyroid gland epithelium and produced brain gene and protein expression profiles that clustered near those of PCBs and PTU, raising doubts on the usefulness of thyroid hormone serum levels to predict and extrapolate environmental contaminants thyroid hormone-related effects.
- While some of the differentially expressed genes can be linked to known molecular mechanisms of neurotoxicity, others open avenues for hypothesis driven research to gain insights on molecular mechanisms of toxicity, to develop biomarkers of exposure/effects, and to test potential ameliorative approaches to mitigate the effects of environmental contaminants.

Objectives

Compare the effects of perinatal exposure to the Northern Contaminant Mixture with the effects of its major components (MeHg, PCBs and OCs) on rat cerebellum and hippocampus gene expression profiles at RNA and protein levels, using microarray and 2D gel electrophoresis.

i) Compare the effects of perinatal exposure to the contaminant mixtures with those of PTU-induced hypothyroxinemia.

ii) Compare the effects of perinatal exposure to PCBs with those of the structurally related PBDEs.

iii) Correlate brain gene expression profile data to systemic toxicity and neurobehavioural outcomes.

iv) Develop and validate novel molecular biomarkers of environmental contaminant exposure/effects.

Introduction

The Northern Contaminant Program (NCP) previously funded our section to perform a neurodevelopmental study on rats perinatally dosed with the "Northern Contaminant Mixture", which was designed by Dr. Wayne Bowers to contain the 27 most abundant pollutants found in the blood of Canadian Arctic mothers. Perinatal exposure to the lowest dose of this mixture (0.05 mg/kg/day) resulted in subtle neurodevelopmental perturbations in rat pups, warranting further investigation (Chu et al., 2003).

Follow-up studies dosed dams with the Northern Contaminant Mixture, or with MeHg, OCs and PCBs administered separately at the same concentration found in the Northern Contaminant Mixture. Effects of perinatal exposure to these mixtures on pups growth, mortality, thyroid hormone levels and neurobehavioural outcomes were monitored (Chu et al., 2004, 2005).

We have applied high throughput methods such as microarray hybridisation and two-dimensional protein gel electrophoresis to monitor gene and protein expression in cerebellum and hippocampus following perinatal exposure to the Northern Contaminant Mixture and sub-mixtures. This report will present and summarize our latest findings, discuss the possible molecular mechanisms underlying toxicity and correlate brain gene and protein expression data to systemic and functional outcomes.

Activities in 2004-2005

PBDE-treated animals were dosed, sacrificed and tissues harvested. Thyroid gland morphology measurements are completed. Proteomic analysis for all the postnatal day (PND) 14 male hippocampus and cerebellum control and high dose treatment groups (including PBDEs) is completed. Differentially expressed proteins were isolated and sent to MALDI-ToF peptide mass fingerprinting for identification. Unfortunately, very few proteins were conclusively identified, and we consequently scaled up the purification procedure in order to isolate larger amounts of protein and facilitate identification. Microarray hybridisation of PND 14 male and female cerebellum and hippocampus for the contaminant mixtures and PTU is completed. However, we ran out of cDNA microarray chips to complete hippocampus and cerebellum gene expression analysis resulting from PBDE exposure. Since this specific cDNA microarray chip was discontinued, we had to switch to Rat whole genome oligoarray chips and re-optimize our labelling and hybridisation protocols. Rat whole genome oligoarray represents a technological advancement over the previously used cDNA microarray with more genes (41,000) and better sensitivity and gene annotation. Microarray

hybridisation of PBDE-treated samples was completed by March 31st, 2006, but data analysis has not been performed yet. A list of differentially expressed genes resulting from exposure to the contaminant mixtures was established and validation of the most interesting genes by real time PCR is underway.

Results

Dams were dosed from gestation day 1 to post-natal day 21 with the Northern Contaminant Mixture (5 and 0.05 mg/kg), or equivalent amounts of the components that constituted the mixture: methyl mercury (2 and 0.02 mg/kg), polychlorinated biphenyls (1.1 and 0.011 mg/kg) and organochlorine pesticides (1.9 and 0.019 mg/kg). Dams were also dosed with DE-71 a technical mixture of polybrominated diphenyl ethers (0.3, 3 and 30 mg/kg body weight). A positive control group for hypothyroxinemia was dosed with propylthiouracil (0.001% PTU in drinking water from GD 6 to PND 21). Subsets of pups were sacrificed on PND 14 and PND 21, corresponding to periods of intense synaptogenesis in the hippocampus and cerebellum (PND 14) and in higher brain areas (PND 21). The effects of such exposures on growth, mortality and thyroid hormone status were reported last year (Chu et al., 2005) and the present report will focus on thyroid gland morphology and brain proteomic (2D gel electrophoresis) and genomic (microarray hybridisation) outcomes.

Thyroid gland morphology

While serum thyroid hormone levels represent a snapshot of thyroid system function at a precise time point, thyroid epithelial thickness may be considered as a record of stresses to the hypothalamo-pituitary-thyroid axis. As expected, the Northern Contaminant Mixture, PCBs and PTU treatment groups that affected serum thyroid hormone levels (Chu et al., 2005) also increased epithelial cell thickness (Figure 1). Despite the fact that exposure to OCs failed to produce measurable effects on serum T4 and TSH levels at post-natal day 14 and 21, it resulted in a mild but statistically significant thickening of the thyroid epithelial layer. This observation, although surprising at first, is corroborated by brain gene and protein expression profiles suggesting that OCs might affect thyroid hormone-dependant pathways in the brain.

Proteomic analysis

Two-dimensional gels were digitalized and protein spots detected with Phoretix software. Samples resulting in 2D gels that consistently failed to reach a minimum number of detected spots (typically one or two per treatment group) were rejected from the analysis. The data from the remaining gels (8-10 per treatment group) were then quantified, normalized, averaged and treatment groups compared. Spots showing differential expression greater than two-fold were inspected manually and reanalysed using different background subtraction and normalisation parameters. Spots consistently showing intensity variation greater than two fold in at least one treatment group were then exported to Genespring GX for statistical and clustering analysis. Figure 2 lists differentially expressed proteins in cerebellum and hippocampus and presents protein expression profiles hierarchical clustering. Based on the data presented in Figure 2, we conclude that:

i) Antagonistic interactions between contaminants occur, as exposure to the Northern Contaminant Mixture produced a pattern that was clearly not the result of a simple addition of the sub-mixtures.

ii) The effects of PCBs and OCs on brain proteome share noticeable similarities. The fact that OCs can stress the hypothalamo-pituitary-thyroid axis (Figure 1) may explain the similarities between OCs, PCBs and PTU-induced hypothyroxinemia.

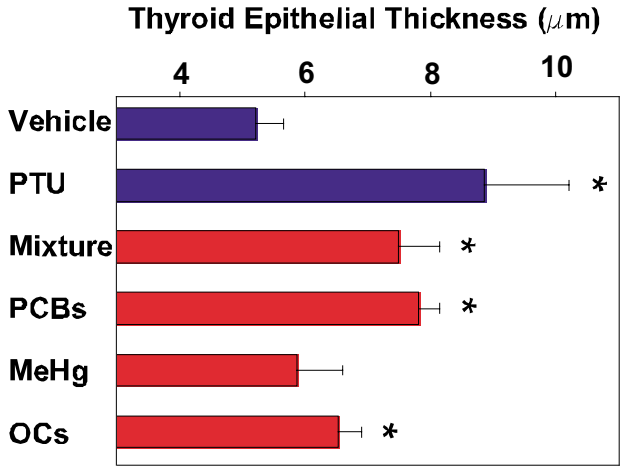


Figure 1. Effects of environmental contaminants on PND 21 pup thyroid gland histomorphology. Measurements of male and female thyroid epithelial thickness are summarized. Error bars represent standard deviation and * indicate statistically significant differences from the control group ($p < 0.05$).

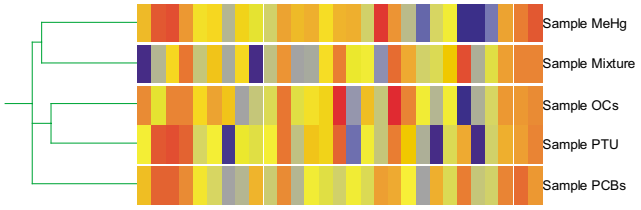
a)

Spot ID	mw	PI	Mixture	MeHg	PCBs	OCs	PTU	PBDE
A2	67	6.75	**2.57**	1.84	**2.09**	**2.26**	**2.99**	1.08
B2	64	6.50	1.99	1.64	**2.10**	**2.58**	**2.63**	-1.08
C2	59	6.60	2.30	2.48	**2.78**	1.81	1.74	1.35
D2	55	6.35	-1.31	**-3.31**	-1.52	-1.41	-1.51	-1.12
E2	47	6.55	**-6.21**	1.33	1.24	**2.04**	-1.05	1.17
F2	42	7.20	1.07	**3.58**	3.00	2.21	**3.93**	-1.94
G2	40	6.15	-1.68	1.04	1.02	1.07	-1.45	**2.63**
H2	36	6.75	1.23	1.08	-1.43	**1.59**	-1.01	-2.49
I2	35	6.50	-2.23	-2.02	**-2.31**	1.20	**-4.92**	
J2	31	5.90	**-8.52**	-1.24	1.38	-1.70	-1.27	1.62
K2	31	6.25	**-1.80**	-1.55	-1.58	-1.38	-1.04	2.41
L2	28	6.65	1.06	1.05	-1.30	1.11	1.09	**3.98**
M2	27	6.55	-1.10	-1.49	-1.37	1.26	-1.11	**2.83**
N2	25	5.35	-2.79	**4.40**	1.70	-1.68	-1.72	
O2	25	6.95	2.83	1.91	1.49	**6.41**	2.42	-4.83
P2	25	6.50	1.52	**-1.90**	1.00	**2.28**	1.17	1.37
Q2	23	6.25	-2.05	**-4.95**	-1.73	-2.16	**-11.82**	
R2	22	6.55	-1.48	**-3.60**	**-2.37**	-1.07	**-2.14**	-7.17
S2	20	5.45	-1.36	-1.44	1.43	-1.99	**-6.98**	-1.37
T2	20	5.90	1.16	-1.06	-1.34	-1.10	**-1.34**	2.01
Total			**4**	**6**	**5**	**6**	**8**	**3**

b)

Spot ID	mw	PI	Mixture	MeHg	PCBs	OCs	PTU	PBDE
A1	64	6.50	**2.05**	2.56	**2.30**	**2.93**	1.37	-1.13
B1	59	6.60	1.16	1.58	1.66	1.95	**2.72**	1.17
C1	38	6.85	-1.03	1.53	**1.62**	1.35	**-3.73**	-1.16
D1	38	6.65	1.24	2.27	1.28	-1.17	**7.15**	-1.63
E1	36	6.75	1.00	1.46	**2.61**	2.30	1.20	1.31
F1	36	6.95	1.30	-1.74	**2.70**	**3.14**	**3.71**	-1.33
G1	36	6.75	1.34	**-2.69**	1.96	1.48	**4.01**	-1.12
H1	31	7.00	-1.91	-1.85	**2.09**	1.62	1.58	-1.46
I1	31	5.70	-1.32	-1.16	-1.33	-1.31	-1.02	**2.25**
J1	30	6.65	-1.07	1.14	1.10	-1.42	**-3.85**	1.11
K1	30	6.30	**-2.08**	1.08	1.24	1.14	**-5.61**	-2.15
L1	25	6.95	1.92	-1.60	**4.86**	4.48	2.46	-1.44
M1	24	6.95	1.05	-1.16	**1.60**	**1.61**	**-3.21**	-1.09
N1	20	5.90	**-3.34**	-1.44	-1.80	-1.34	**-5.66**	
Total			**3**	**1**	**7**	**3**	**9**	**1**

c)



d)

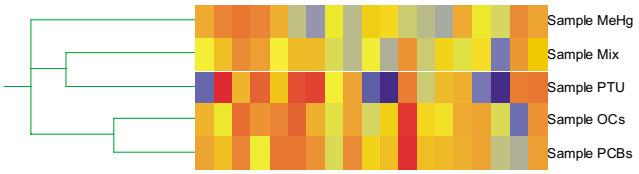


Figure 2. Differentially expressed protein in the A) cerebellum and B) hippocampus of PND 14 male pups. Gene expression in the control group was arbitrarily set to one and up or down-regulations greater than two-fold are highlighted in green or yellow. Statistically significant changes are indicated in bold numbers. C) and D): Hierarchical clustering of the data presented in A) and B).

iii) Hierarchical clustering analysis shows that methyl mercury and Northern Contaminant mixture treatment groups cluster on the same branch in the cerebellum, a known target of MeHg neurotoxicity (Fonnum and Lock, 2000).

iv) Comparison of PCBs (1.1 mg/kg) and PBDEs (30 mg DE-71/kg) exposure effects on brain protein expression pattern suggests a lower *in vivo* potency for PBDEs.

Of the differentially expressed proteins listed in Figure 2 A) and C), five were differentially expressed in both cerebellum and hippocampus, of which one was successfully identified as Protein Disulfide Isomerase A3 by MALDI-ToF peptide mass fingerprinting. PDI has chaperonin functions and is involved in protein folding and disulfide bridge formation (Wang, 1998). Interestingly, MeHg has a high affinity and covalently binds to reduced cysteine sulfhydryl group (Ballatori, 2002). We did not observe a change in PDI total protein expression but rather a probable shift in its phosphorylation state. It is however difficult to predict the functional outcome of this phosphorylation shift, as very little information is available from the literature on the effects of post-translational modifications on Protein Disulfide Isomerase activity, sub-cellular localisation and binding partners.

The identity of two other proteins will need to be reconfirmed as we failed to reach the threshold for their unambiguous identification by MALDI-ToF peptide mass fingerprinting. The first tentatively identified protein is involved in oxidative stress response and the other is a biomarker of neuronal differentiation. Once their identity is confirmed, these two proteins may be good biomarkers of environmental contaminant exposure and effects.

Identification of differentially expressed proteins from brain samples has been more difficult than expected. We are now developing a protein pre-fractionation protocol to improve protein yield and purity, as it should facilitate unambiguous protein identification by MALDI-ToF peptide mass fingerprinting.

Microarray hybridisation

Total RNA was isolated from the cerebellum and hippocampus of PND 14 male and female pups and

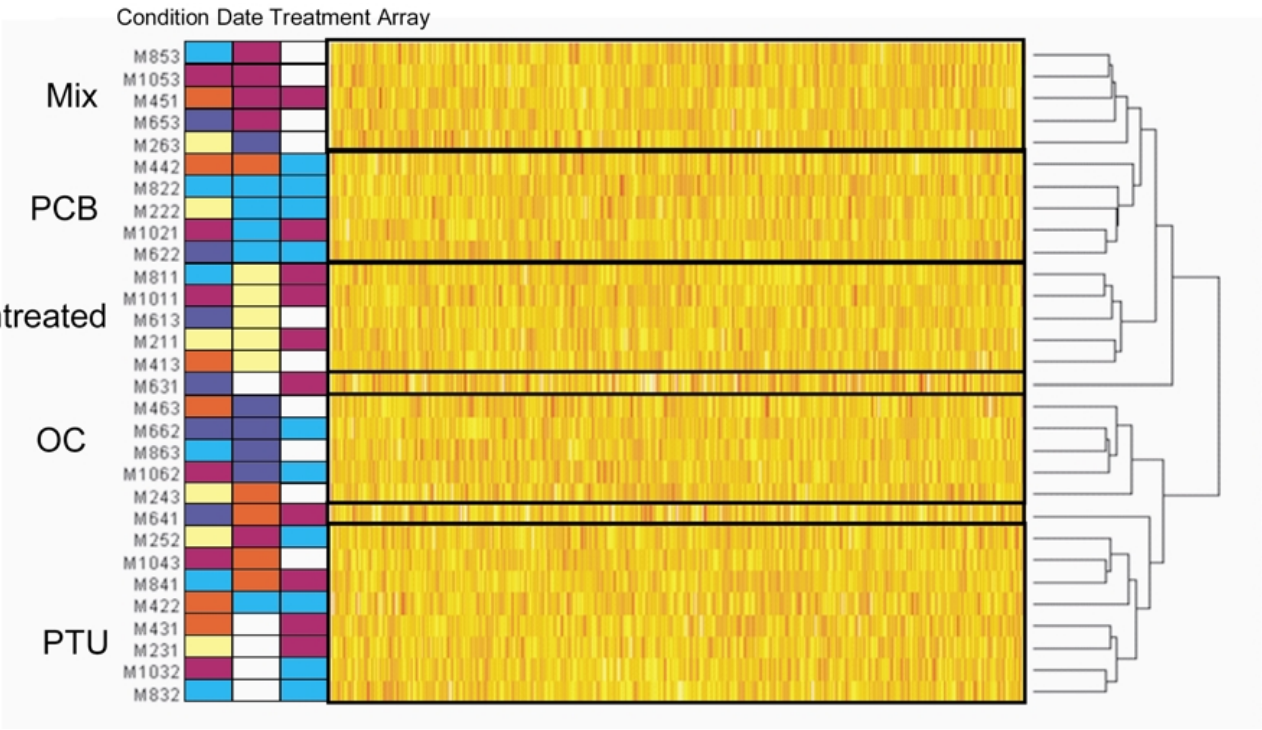


Figure 3. Hierarchical clustering of gene expression data from PND 14 male cerebellum. Coloured squares on the left side represent treatment groups (MeHg treatment group is indicated in red).

RNA integrity assessed by Agilent Bioanalyzer. Five male and five female biological replicates per treatment group were hybridized to Agilent cDNA microarrays, using Rat Universal Reference RNA as a common reference. Unfortunately Agilent cDNA microarray chips were discontinued and in order to complete the analysis of PBDE treated samples, we had to switch to oligoarray chips and optimize new labelling and hybridisation protocols. Oligoarray hybridisation of PBDEs-treated samples has just been completed and gene expression data analysis will soon be performed.

Figure 3 presents the hierarchical clustering of gene expression profiles in PND 14 male cerebellum. Despite the fact that the same pollutants were present in the Northern Contaminant Mixture and submixtures and that a limited number of genes presented statistically significant differential expression, unsupervised clustering resulted in most samples grouping according to their treatment group (with the exception of MeHg-treated samples that clustered on two separate main branches). The similarity between the effects of OCs and hypothyroxinemia observed in brain protein patterns also seems to be confirmed by this analysis as OCs-treated samples clustered on the same branch as PTU treatment group.

Differential expression of a few genes was shared in both cerebellum and hippocampus, and/or in both male and female pups. Interestingly, a few of those genes can be linked to known neurotoxicant mechanism of toxicity, such as transthyretin, involved in thyroid hormone binding and transport (Chauhan et al., 2000), myelin binding protein, a previously characterized biomarker of hypothyroidism (Zoeller et al., 2002), and PCD-5 and Tspan-5, two biomarkers of Purkinje cells maturation (Rong et al., 2004; Juenger et al., 2005). Other genes, such as cysteine-sulfinate decarboxylase, a rate limiting enzyme in taurine biosynthesis (Schuller-Levis and Park, 2003), and SC1, an extra-cellular matrix glycoprotein enriched during development and injury (Mendis et al., 1996) may provide new insights on the molecular mechanisms of neurotoxicity. Validation of gene expression data by real time PCR is already underway and will allow more precise quantification, measurements in more biological replicates and at different dose levels.

Discussion and Conclusion

The data reported here form a surprisingly cohesive picture with the effects of the Northern Contaminant Mixture on a wide array of endpoints.

Data on growth and survival, neurobehaviour, neurochemistry and hepatic and endocrine functions (Chu et al., 2004, 2005; Desaulniers et al., 2005) all uncovered significant interactions between compounds present in the Northern Contaminant Mixture and suggested that while some toxicological endpoints could be attributed to specific components of the Northern Contaminant Mixture, others could not be accurately predicted from the list of individual pollutant.

In this study, gene expression profiles clustered reasonably well according to the samples treatment group, but the expression of specific genes resulting from exposure to the Northern Contaminant Mixture could not be accurately predicted from the list of its components. Consequently, biomarkers developed in single compound exposure studies might be of limited use in the context of exposure to complex mixtures. Similarities between protein and gene expression profiles resulting from OCs, PCBs, and PTU exposures may have implications for risk assessment, as they suggest that serum thyroid hormone levels might be poor biomarkers of environmental contaminant thyroid hormone-related effects.

Behavioural impairments resulting from perinatal exposure to the Northern Contaminant Mixtures or sub-mixtures have been characterized (Chu et al., 2003, 2004), which will allow correlation of gene expression data to functional neurological outcomes. For example, OCs had very little if no effects on behaviour and therefore, differentially expressed genes shared by PCBs and OCs treatment groups are unlikely to be good biomarkers of behavioural impairments (but may still be good broad-range biomarkers of exposure). Another example is MeHg-induced hyperactivity that was not found to be affected by co-exposure with PCBs and OCs. Consequently, differentially expressed genes or gene patterns shared by the Northern Contaminant Mixture and MeHg may be good biomarkers of hyperactivity. Such analysis will be further refined as we will validate biomarkers.

The high throughput genomic and proteomic approaches used in this study provided us with a list of differentially expressed genes to be validated by real time PCR. While some of the differentially expressed genes can be linked to known molecular mechanisms of neurotoxicity, other genes open avenues for hypothesis driven research to gain insights on molecular mechanisms underlying toxicity, to develop biomarkers of exposure/effects, and to test potential ameliorative approaches to mitigate the effects of environmental contaminants.

Expected Completion Date

October 2006

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Long-Term Effects Following Postnatal Exposure to Breast Milk Contaminants: Is it Real?

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Abstract

Our goal is to assess if the magnitude of the health risks associated with postnatal exposure to environmental contaminants is negligible relative to the health risks associated with *in utero* exposure. To achieve this a two step research strategy was adopted. First, an understanding of the toxicity of the components (methylmercury, PCBs, organochlorine pesticides) from a complex mixture of "Northern Contaminants" (NCM) is being derived by analysing samples from other NCP projects testing the effects of combined *in utero* and lactational exposure to the NCM. Based on these findings, the second step is to conduct a cross-fostering experiment to characterize if the effects can be attributed to the exposure received *in utero*, or from lactational exposure during the postnatal period. The current report summarizes important results of the first step of the research strategy. Pregnant rats were dosed each day from gestation day 1 to postnatal day (PND) 21, with either corn oil (control), or two dose levels of 12 organochlorine pesticides (OP), methylmercury (MeHg), 14 polychlorinated biphenyls (PCBs), or a mixture (NCM) including all these chemicals. The low dose groups of MeHg, PCBs, and NCM, increased the mRNA expression of the hepatic detoxification enzyme, cytochrome P450 (CYP) 1A1, whereas all high dose groups increased mRNA levels for CYP1A1, 1B1, and 2B1. These also act as hydroxylase enzymes on steroid hormones, and the high dose treatments (except for MeHg) increased the hepatic production of the toxic catecholestrogen, 2-hydroxy-estradiol-17β (2-OH-E2). The latter is detoxified by being metabolized into the beneficial 2-methoxy-E2 (2-MeO-E2) by the enzyme catechol-*o*-methyltransferase (COMT), however the activity of COMT could not compensate for the high production of 2-OH-E2. DNA methylation is one of the mechanisms that regulates gene expression, cell differentiation during development and disease, chromosome structure and stability. Effects on this system constitute key findings. The high dose PCB treatment decreased the hepatic mRNA abundance for DNA methyltransferase-1 (DNMT1), -3a and -3b, while the high dose MeHg reduced mRNA levels for DNMT1, and DNMT3b. The OP, and surprisingly the NCM treatments, had no effects. These major changes highlight chemical specific effects, and could be early indicators of long-term adverse effects on liver function, and perhaps other estrogen dependent tissues.

Key Messages

- In the rat model, exposure of the fetus to high doses of contaminants during pregnancy, combined with exposure received by the pups through the mothers milk, dramatically changed the normal transformation of the hormone estrogen by the liver. The outcome is the production of toxic estrogens (catecholestrogens) in excess

of the beneficial one (2-methoxyestrogens). This could be an early indicator of long-term adverse effects on liver function.

- Our genetic code is included in the DNA located in the chromosomes of our cells. The genetic code is constantly used by cells for normal functions and differentiation into various tissue types during development and disease progression. A group of proteins found in our cells, called DNA methyltransferases (DNMTs), add methyl groups to the DNA. This is a normal and vital process that controls access to the genetic code, and confers the normal structure and stability of our chromosomes. Exposure to PCBs, and MeHg (but not OPs), reduces the amount of cellular messages required for the production of these DNMTs, suggesting that the genetic code could become abnormal, and that the cells may be at risk of not functioning and developing normally.

Objectives

i) Assess if endocrine disruption involving estrogen metabolism, or early changes in DNA methylation, could be used as indicators of adverse health effects.

ii) Derive an understanding of interactions among chemical families in the toxicity of contaminant mixtures.

iii) Test if the magnitude of the health risks associated with postnatal exposure to environmental contaminants is negligible relative to the health risks associated with *in utero* exposure (this represents the activities of the current year).

Introduction

Investigations in wildlife revealed numerous examples of adverse health effects resulting from exposure to environmental contaminants (ECs). These concepts raised concerns that human health could also be threatened by exposure to ECs. Some ECs include persistent organic pollutants (POPs) that accumulate in fatty tissues of animals and humans. Given that breastmilk is rich in fat, it is during the postnatal period, while breastfeeding, that humans receive some of the highest exposure levels to POPs. It is also known that the benefits of breastfeeding far outweigh putative risks in the general population. However, for reasons that are not fully understood, and which may include differences in detoxification enzyme activites or unusual exposure to ECs, some individuals have abnormally high levels of ECs in their body. The absence of a risk for these individuals is more difficult to ascertain. Northern communities have a higher body burden of some ECs and may be at a higher risk of adverse health effects than Southern populations. Government agencies must protect adults and infants from unacceptable exposure to ECs, but there is little information about the level of exposure during the postnatal period that could increase health risks above those already induced by exposure of the fetus in the womb. No interventions can prevent exposure of the fetus during pregnancy. However, in the cases of mothers with an unusually high body burden of ECs, if exposure during the postnatal period is responsible for significantly increasing health risks, it could be valid to decrease postnatal exposure by reducing breastfeeding intensity during specific critical phases. The determination of an unacceptable exposure level to ECs in infants, and preventing the exposure from occurring, are complicated by the difficulty in identifying the culprit chemical(s) from the mixture of chemicals to which humans are exposed, and also by the fact that the abundance of ECs are not the same *in utero* and during the postnatal period. For example, the infant body burden of methylmercury (MeHg) declines postnatally whereas that of persistent organochlorines increases. Therefore, the *in utero* and postnatal periods are distinct developmental phases with regard to chemical exposure, and also in relation to tissue sensitivity and detoxification capacity.

The current project aims to assess the additive effects contributed by lactational exposure, above those already attributed to the exposure to ECs occurring *in utero*. Classical indicators of toxicity will be compared to endpoints that are important determinants of developmental processes. Estrogen metabolism and signalling are major regulators of brain development. Estrogens affect mood behaviors, numerous systems (ovaries, uterus, placenta, testicles, breast, bones, vascular system, hypertension, lipid metabolism, immune system) and cancers (breast, endometrium, ovary, prostate, liver, kidney, brain) in both the male and female. DNA methylation is one of the epigenetic modifications involved in chromosome stability, chromosome X inactivation, modifications in centromere structure, genomic imprinting,

gene expression and tissue differentiation at the appropriate time during development. Studying these endpoints has great potential to identify new bioindicators of health effects, and improve the tools in risk assessment strategies.

Activities in 2005-2006

Animal treatments and origin of samples
To fulfil objectives 1 and 2, and generate new information on the toxicity of "Northern Contaminants", samples were collected from two projects (NCP-H05: Bowers WJ, Nakai J, Chu I, et al.; NCP-H17: Pelletier G, Wade M, Chu I, et al.) testing effects of a chemical mixture (and its components) developed by Dr. Bowers. Analyzing these samples was also required to determine the relevant OPs and MeHg dosages to be tested in the cross-fostering experiment. In both experiments, pregnant rats were dosed each day from gestation day 1 to PND21, with either corn oil (control), or two dose levels of 12 OPs (1.9 or 0.019 mg/kg/day), MeHg (2, or 0.02 mg/kg/day), 14 PCBs (1.1 or 0.011 mg/kg/day), or a mixture including all these chemicals (5, or 0.05 mg/kg/day).

Selection of Endpoints and techniques
ECs could have adverse effects on cell function by themselves, or because of indirect effects as shown here. Many ECs induce enzymatic reactions that lead to their elimination and excretion. The addition of hydroxyl groups to the contaminants by the cytochrome P450 (CYP) family of enzymes is often the initial step in the elimination pathway. The activation of these pathways by ECs could lead to indirect adverse effects by favoring the abnormal hydroxylation of other substances present in our body such as the estrogens, a family of steroid hormones. Some hydroxylated estrogens, called catecholestrogens, are toxic and must be methylated by the enzyme catechol-*o*-methyltransferase (COMT) to be detoxified. Some ECs inhibit , or decrease the expression of COMT in the liver and the brain . Collectively, these observations suggest that exposure to ECs could lead to tissue specific accumulation of toxic estrogen metabolites. Therefore, we measured the mRNA expression of many CYPs (CYP 1A1, 1B1, 2B, 3A) as classical indicators of exposure, whereas COMT mRNA expression, as well as estrogen receptor α, and estrogen metabolites, were measured as indicators of effects. The production of the estrogen metabolites was assessed by measuring the hepatic microsomal transformation of ^{14}C-E2 by high performance-thin layer chromatography (HP-TLC) and phosphorimaging. This method assesses effects on the production of 13 known estrogen metabolites, and also unidentified radioactive metabolites. Another analytical method is being developed to measure the most important native metabolites directly in the tissue (Dr. Nanqin Li).

Abnormal DNA methylation has been associated with changes in gene expression, a number of cancers, infertility, developmental, neurological, immunological, and age-related disorders. DNA methylation reactions are catalyzed by families of DNA methyltransferases (DNMT1, 2, 3) with important ones being DNMT1, 3a, and 3b. We demonstrated that aryl hydrocarbon receptor-agonists (3 non-*ortho* PCBs, 6 polychlorinated dibenzodioxins, and 7 polychlorinated dibenzofurans) decreased the mRNA expression of DNMT1 in the rat brain and liver. Therefore, real time RT-PCR is being used to monitor the mRNA expression of DNMT1, 3a, and 3b. The magnitude of the changes in DNA methylation is suggested to progress from changes in specific gene promoters, then DNA repeated sequences, and/or global DNA methylation, but it remains uncertain if changes in DNMT expression are required to induce aberrant DNA methylation. Consequently, first, methylation specific (MS)-PCR was used to investigate the promoter regions of specific genes for which the expression was reduced by the treatments. Second, the methylation status of the retrotransposon Long Interspersed Nuclear Element-1 (LINE-1) has been analyzed by methylation specific PCR, sodium bisulfite treatment and methylation sensitive restriction enzymes (*Hinf1*, *Mbo1*). This abundant DNA repeated sequence is normally methylated thereby preventing its expression and chromosome instability. Third, a method is being developed to assess effects of treatment on global DNA methylation .

Effects on brain are being investigated, using immunohistochemistry to visualize regional differences in protein expression for estrogen dependent endpoints, and indicators of neuronal development. Next, the critical brain regions expressing these endpoints will be investigated quantitatively using real time RT-PCR and Western blot analysis.

Discussion

Three series of significant effects of the Northern Contaminants in female rats are reported in the following 3 paragraphs. The first series refers to

changes in mRNA abundance for CYPs at PND29. The mRNA expression of CYP1A1 was increased in the low dose PCB, MeHg, and NCM, but not in the OP treated group. At high dose of exposure synergistic increases in mRNAs for CYP1A1, 1B1, and 2B1 were observed in the NCM group relative to other high dose groups. Given the low dose effects and the synergic increases in CYP1A1 and 1B1 mRNAs, these normal detoxification responses represent the most sensitive indicators of exposure in this study.

The second series relates to the important changes in the metabolism of estrogens at PND21. Since CYP1A1 and CYP1A2 are the main P450 enzymes responsible for 2-hydroxylation of estrogens in rat liver, we found significant increases in the production of the toxic catecholestrogen 2-OH-E2, which occurred in the OP, the PCB, and the NCM, but not the MeHg high dose group. 2-OH-E2 is methylated by the enzyme COMT into non-toxic methoxyestrogens, among which 2-methoxy-E2 was found to have numerous protective effects against cardiovascular and renal diseases , and cancer . In control rats, the production of the toxic 2-OH-E2 (0.19 ± 0.06 pmol/mg/min) was greatly counteracted by its rapid conversion into two different forms of methoxyestrogens, the beneficial 2-methoxy-E2 (0.43 ± 0.09 pmol/mg/min), and 2-OH-3-methoxy-E2 (0.31 ± 0.07 pmol/mg/min), for a total production of methoxyestrogens of 0.74 ± 0.17 pmol/mg/min. In this control group, the ratio for the production of 2-OH-E2/total methoxyestrogens is 0.45 ± 0.14, which demonstrates protection against the production of 2-OH-E2. In contrast, this ratio for the OP, PCB, and NCM group, is 4.28 ± 1.44, 6.24 ± 2.38, and 4.1 ± 1.5, respectively, demonstrating that the large production of 2-OH-E2 could not be counteracted by the activity of COMT. High doses of OP, PCB, and NCM also increased the amount of CYP1B1 mRNA. This is the main enzyme that catalyzes 4-hydroxylation of estrogens. 4-OH-E2 is the most toxic and carcinogenic catecholestrogen. Fortunately, its production and transformation into methylated forms were not significantly altered. Our results clearly demonstrate that the high dose treatments greatly modified the hepatic metabolism of estrogens to favour the accumulation of the catecholestrogen 2-OH-E2, and likely the consequential production of reactive oxygen species, lipid peroxidation, adduct formation, and initiation of carcinogenesis. The cyclical exposure to endogenous estrogens in female rats exposed to PCBs and AhR-agonists, has been provided as an explanation for the higher rate of hepatic cancers in female than male counterparts. Similar tissue-specific enzymatic imbalances could occur in other estrogen tissue targets (e.g.: brain, reproductive system, breast).

Finally, DNA methylation is a new area of investigation in toxicology. At PND29, the PCB treatment decreased the mRNA levels for all DNMTs investigated (DNMT1, 3a and 3b), MeHg decreased DNMT1 and 3b, whereas the OP and NCM treatment had no effects. These observations raise the possibility of altered DNA methylation, and this was confirmed by MS-PCR analysis suggesting that the promoter region of DNMT3b was hypermethylated in at least the PCB group. Similar hypermethylation results were obtained for $p16^{ink4a}$, but not for DNMT1, 3a, or LINE1. Global changes in DNA methylation is usually a latter event and this will soon be investigated. Hypermethylation of DNMT3b and $p16^{ink4a}$, are consistent with their reduced mRNA expression. Changes in DNA methylation, with global hypomethylation and site specific hypermethylation, are landmarks in the progression of carcinogenic events. A decrease in $p16^{ink4a}$ expression is common in various cancers and suggests increased mitotic activity with less time for DNA repair. Our experiment was too short to determine if indeed these treatments would have led to hepatic neoplasia. The DNA methylation system appears to be a toxicity target for PCBs, and MeHg, but not for OPs. These changes might form the basis for a variety of toxic outcomes, and given the stability of aberrant DNA methylation, may become evident only later in life.

In conclusion, the change in CYP1A1 mRNA expression was the most sensitive endpoint in this study. However, while increases in CYP1A1 expression is a normal detoxification response, effects on DNMTs and on estrogen metabolism are abnormal events and require further dose-response investigations. The current results support that changes in DNA methylation, estrogen metabolism and signaling, are targets of some Northern Contaminants, and could be the origin for some long-term adverse health effects. We still cannot assess if the magnitude of the health risks associated with postnatal exposure to environmental contaminants is negligible relative to the health risks associated with *in utero* exposure, but this will be investigated in the

current year using a lower dose of exposure. This will also complement a dose-response investigation on DNA methylation and estrogen metabolism endpoints.

Expected Project Completion Date

March 2007.

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Exposure To Heavy Metals And Persistent Organic Pollutants In Nunavik: The Nunavik Health Study (Human Health)

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Abstract

The present study has been conducted in Nunavik in 2004-2005 within the framework of The Nunavik Health Survey (Qanuippitaa), during which recent information regarding the health of the Inuit population of Nunavik was gathered. The objectives of this study are 1) to investigate changes in environmental contaminant exposure among the Inuit of Nunavik by updating exposure assessment, traditional/country food consumption and protective nutritional factors intake and 2) to begin monitoring emerging environmental contaminants. Updated information on contaminant exposure can be used as a basis for informed decisions about food use by the Inuit of Nunavik.

Key Messages

- Decreasing levels of mercury and lead are observed in men and women of all age groups since 1992 and are mainly attributable to changes in dietary intake and to the ban on lead shots used for hunting. Mean levels are now below WHO TDIs, but levels still exceed these limits for few individuals.
- Exposure to mercury is mainly related to the consumption of marine mammal and country fish, while exposure to lead is related to the consumption of game birds.
- Decreasing levels of cadmium are observed in men and women aged 25 years and over, but levels remained the same in men and women aged 18-24 years. Exposure is mainly attributable to smoking.
- Data for persistent organic pollutants remain to be completed before final analyses.

Objectives

This study is nested in the Qanuippitaa survey that took place during Fall 2004. The main objective of

Qanuippitaa is to evaluate the health status of the Inuit of Nunavik, and this includes an important component addressing exposure to environmental contaminants and its effects on human health, especially chronic diseases. The current study addresses the issue of exposure assessment in the Qanuippitaa survey but the evaluation of the effects of exposure on chronic diseases is beyond the scope of the current report. The specific objectives of the study presented here are:

i) To assess changes in contaminant exposure among the Inuit of Nunavik during the past decade by updating information available on contaminant levels measured in adult blood, on traditional/country food consumption and on protective nutritional factors during the 2004 Nunavik Health Survey, and comparing this information with data collected in 1992 during the Santé Québec Health Survey.

ii) To begin the monitoring of emerging contaminants of concern in northern regions.

Introduction

Human exposure to environmental contaminants is a well known phenomenon in the Canadian Arctic. The Inuit of Nunavik are exposed to a plethora of toxic substances that are carried from southern to northern latitudes by oceanic and atmospheric transport and biomagnified in arctic food webs. As the Inuit traditional diet comprises large amounts of tissues from marine mammals, fish and terrestrial wild game, the Inuit are more exposed to heavy metals and persistent organic pollutants (POPs) than populations living in southern regions. Heavy metals of concern include mercury (Hg), lead (Pb) and cadmium (Cd) and other contaminants of concern are POPs.

Hg is an environmental contaminant originating from both anthropogenic and natural sources. Despite significant reduction of Hg emissions in Europe and North America, Hg levels are still high in the Arctic (AMAP. 2002b). Although most of the Hg released in the environment is inorganic or elemental, it can accumulate in the water where it can be transformed into methyl mercury (MeHg) by microbial action. This highly toxic form of Hg is accumulated in animal tissues, especially in the liver and the kidney, and is biomagnified in the food chain (Hansen and Gilman. 2005). The most important sources of human exposure to MeHg are fish and marine mammals consumption (Bjerregaard and Hansen. 2000; Dewailly, et al. 2001; Grandjean, et al. 1992; Hansen and Gilman. 2005; Van Oostdam, et al. 2005). MeHg mainly affects the nervous system and can cause paresthesia, ataxia and tunnel vision (ATSDR. 1999b). It is also toxic to the kidney, liver, reproductive organs, and the cardiovascular system (ATSDR. 1999b). Foetal and infant exposure to MeHg via transplacental and lactational routes (ATSDR. 1999b) is of great concern regarding neurodevelopment.

Most of the Pb in the environment comes from anthropogenic sources and is carried in the Arctic by atmospheric transport (AMAP.2002b). It has been clearly shown that Pb environmental levels are decreasing in Arctic regions since the ban of leaded gasoline gasoline (AMAP. 2002b; Van Oostdam, et al. 2005). However, high levels of Pb can still be found in Inuit populations in certain Arctic regions due to the past and/or present use of lead shots for harvesting wild game (AMAP. 2002b; Dewailly, et al. 2001; Levesque, et al. 2003). In 1999, the use of lead cartridges was banned in Canada for the hunting of migratory birds, and the public health authorities of Nunavik actively informed the population about Pb (Levesque, et al. 2003) in order to reduce lead shot use and Pb exposure. The most critical effect of Pb at low concentrations is reduced cognitive development and intellectual performance in children (WHO. 2004). Pb principally affects the brain and nerve tissues, but can also cause kidney damage and dysfunction, anaemia, intestinal dysfunction, and reproductive problems (ATSDR. 2005).

Cd is released in the environment from both anthropogenic and natural sources and it can accumulate in lichen and vegetation (Crete, et al. 1989; Nash III and Gries. 1995), which is then eaten by the caribou and moose (Robillard, et al. 2002). Cd accumulates in the soft tissues rather than in the muscle or fat and it is typically higher in the kidney than in the liver (Robillard, et al. 2002). However, studies have shown that human exposure to Cd is most often attributable to tobacco smoking. The major health risk associated with Cd is nephrotoxicity (WHO. 2004). Chronic exposure can also cause anaemia, disturbed calcium and vitamin D metabolism, bone loss and cardiovascular diseases (ATSDR. 1999a).

The traditional suite of POPs comprises polychlorinated dibenzo *p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and chlorinated pesticides. Due to their lipophilic properties, these compounds accumulate in fatty tissues. They are also found in human milk and are able to cross the placental barrier. Several studies have shown that *in utero* exposure to PCBs may have detrimental effects on several aspects of cognitive development (Darvill, et al. 2000; Jacobson, et al. 1990; Patandin, et al. 1999; Stewart, et al. 2003; Walkowiak, et al. 2001). Some of these organic compounds have endocrine disrupting properties and have the capacity of altering natural hormone signalling pathways such as thyroid hormones hormones (Brouwer, et al. 1998; Cheek, et al. 1999; Lans, et al. 1993), estrogens and androgens (Bonefeld-Jorgensen, et al. 2001). POPs have also been found to be carcinogenic, hepatotoxic and have reproductive and immunotoxic effects effects (Safe. 1994; Schecter, et al. 2006; Schmidt. 1999). The Inuit are exposed to these compounds through the consumption of contaminated traditional foods, particularly seal and beluga fats. Recently, declining trends in POPs' body burdens were observed among Inuit newborns newborns (Dallaire, et al. 2003a) and Caucasians newborns residing along the Lower North Shore of the St. Lawrence River (Dallaire, et al. 2002a). Similar observations were reported among Arctic wildlife species (Braune, et al. 2005).

Although concentrations of organochlorines in human, wildlife and environmental matrices are decreasing, other compounds manufactured in industrialized regions of North America and Europe have been recently measured in environmental media, in tissues of wildlife species and in human tissues from the Arctic. The latter emerging POPs include perfluorooctanesulfonate (PFOS), halogenated phenolic compounds (HPCs) and brominated flame retardants such as polybrominated diphenyl ethers (PBDEs). PBDEs are a class of brominated flame retardants that are extensively used in various industrial and commercial products such as textiles and electronic devices (Alaee, et al. 2003). They share similar physical and chemical properties with PCBs, PCDD and PCDF, which suggest that they may induce similar detrimental developmental, hormonal and reproductive effects (Birnbaum and Staskal. 2004; Gill, et al. 2004; Hooper and McDonald. 2000; McDonald. 2002).

PFOS is an end-product of fluorochemicals that have been produced since 1970 but have received less attention until now due to the difficulty in analytic measurements (Hekster, et al. 2003). Animal studies have demonstrated that PFOS may alter thyroid hormone homeostasis, affect fatty acid transport and metabolism as well as membrane function (Lau, et al. 2004; Thibodeaux, et al. 2003). The presence of PFOS in biological matrices was reported in several wildlife species of the Arctic (Bossi, et al. 2005; Giesy and Kannan. 2001; Smithwick, et al. 2005; Tomy, et al. 2004).

Halogenated phenolic compounds that include metabolites of PCBs (hydroxylated and methylsulfone), pentachlorophenol and others chlorophenols have also been recently measured in environmental media and tissues of wildlife from circumpolar regions (Sandala, et al. 2004; Verreault, et al. 2005). Significant amounts of some of these compounds have been detected in the blood of Inuit adults (Sandau, et al. 2000) and in cord blood from Inuit newborns in Nunavik (Sandau, et al. 2002). In the latter study, significant associations were reported between thyroid hormone level and a composite measure of PCP and hydroxylated-PCBs metabolites. HO-PCB and PCP have been shown to strongly inhibit thyroid hormones sulfation in in vitro studies (Schuur, et al. 1998).

Given the efforts to reduce exposure of Northern Aboriginal people to heavy metals and POPs during the past decade, periodic re-assessment of exposures are needed to evaluate the efficiency of implemented programs and information campaigns (i.e. ban of lead shots, earlier communication campaigns, etc.). As several new compounds sharing similar properties with POPs are entering the Arctic environment and its food webs, it is of prime importance to assess their levels in the Inuit in order to provide up-to-date information to the population and propose effective and viable public health advices respectful of the traditional lifestyle. Therefore, a research theme in the Nunavik Health Survey 2004 was dedicated to the comparison of current exposure to environmental contaminants with that assessed in the 1992 Santé Québec Health Survey. This component of the program also aims at evaluating the effects of exposure on chronic diseases, but this aspect is not covered by the study presented in the current report.

Activities in 2005-2006

The Nunavik Health Survey was conducted in collaboration with the Québec National Institute of Public Health, the Nunavik Regional Board of Health and Social Services and the Statistics Institute of Québec. Data collection was carried out between August 30th and October 1st, 2004 on the icebreaker and scientific research vessel CCGS Amundsen. Our team visited the 14 communities of Nunavik (http://www.qanuippitaa.com/) and recruited 917 participants.

People were invited to participate in the study and written informed consent was obtained from all participants, who were then invited aboard the Amundsen for data collection. Information was gathered in three stages: two face-to-face meetings, and one clinical session. The first face-to-face interviews were conducted to collect information on socio-demographic characteristics, environmental factors, lifestyle habits (diet: actual country food versus store-bought food intakes, smoking, alcohol consumption, exercise, drug use), cultural dimensions of health, physical and psychological health status, knowledge and perceptions of health risks related to contaminants in country foods, level of participation in land based activities (hunting, fishing, etc.), and main causes of morbidity. Then a clinical session was conducted involving physiological and anthropometric measurements such as blood sampling (for analysis of blood lipids, insulin and glycaemia, contaminants, iron status among women), blood pressure, height, weight, waist and hip measurement, and dental examination. Finally, another face-to-face interview was conducted by a nurse to administer a 24-hour dietary recall and a food frequency questionnaire.

A total of 1056 individual have signed a consent form. Among them, 917 persons aged between 18-74 years old have filled the individual questionnaire and have accepted to receive a blood puncture, resulting in a participation rate of 69.6%. For the food frequency questionnaire, participation rate was 67.3% for a total of 778 participants.

Concentrations of mercury, lead and cadmium were measured during year 2004-2005. In 2005-2006 167 samples were analyzed for persistent organic pollutants (POPs) that were also determined during the 1992 Santé Québec Health Survey. Plasmatic concentration of new halogenated hydrocarbons such as PBDEs, PFOS, hydroxy-PCBs, methyl-sulfone PCBs and chlorophenols were determined for the same participants. Determination of organic compounds is still underway and his planned to terminate by the end of 2006.

Data on food and nutrient intakes was obtained using a food frequency questionnaire and a 24-hour dietary recall. The food frequency questionnaire was administered to women and men and measured their consumption of traditional/country food for all four seasons during the year before the survey. Traditional/country food refers to food items derived from fishing and hunting (including several parts of marine mammals such as meat, fat, skin, liver). The list of items about traditional/country foods is more exhaustive than that used in 1992. Additional questions regarding the consumption of specific foods were asked to the participants in order to document some Inuit prevalent nutritional deficiencies (vitamins A and C, folic acid, calcium, iron). Hence, information about the consumption frequency of fruits, vegetables, commercial meats, dairy products and whole-grain products was collected in the food frequency questionnaire.

The 24-hour dietary recall will permit to calculate the mean and median intakes of energy and nutrients. The contribution of foods or food groups to nutrient intakes, the importance of traditional/country foods and store-bought foods will also be assessed and this, according to socio-demographic factors. Comparisons with the 1992 Health Survey will also be done. Finally, questions about food security, accessibility, cost and taste, personal values, dietary attitudes, perceptions and beliefs of traditional/country foods and store-bought foods, practice of hunting and fishing will also be included in order to better understand Inuit dietary intakes.

Laboratory analysis:
Laboratory analysis for contaminants was performed at the human toxicology laboratory of the "Institut National de Santé Publique du Québec" (INSPQ), which is accredited by the Canadian Association for Environmental Analytical Laboratories and accredited ISO 17025. Their expertise in the determination of heavy metals and persistent organic pollutants in human fluids and tissues is recognized internationally. This laboratory participates in the QA/QC program of NCP.

In 1992, blood mercury concentrations were determined by cold-vapour atomic absorption spectrometry at the human toxicology laboratory of the INSPQ. This analytic method has a detection limit of 1 nmol/L. Blood lead and cadmium concentrations were measured by atomic absorption spectrometry (graphite furnace). Detection limits were 0.05 µmol/L for lead and 0.2 µg/L for cadmium. For the 2004 Nunavik Health Survey, blood mercury, lead and cadmium concentrations were measured by ICP-MS (Inductively coupled plasma mass spectrometry). Accuracy and precision were measured using reference materials from the Toxicology Laboratory of INSPQ's Interlaboratory Comparison Program.

During the 1992 Santé Québec Health Survey, the traditional suite of persistent organic pollutants (PCBs IUPAC #: 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187, aldrin, α-chlordane, γ-chlordane, cis-nonachlor, *p,p'*-DDE, *p,p'*-DDT, hexachlorobenzene, mirex, oxychlordane, transnonachlor, β-HCH) were measured in plasma according to the following method; blood samples (10 ml) collected in vial containing EDTA were centrifuged (10 min, 5 000 rpm) and the plasma was transferred in glass vials pre-washed with hexane. Samples were thawed overnight at 4°C and a 2-ml aliquot was extracted with hexane. The lipid extract was then cleaned-up on Florisil columns and taken to a final volume of 100 µL. Organochlorines were quantified on a HP-5890 series II gas chromatograph equipped with dual-capillary columns and dual Ni-63 electron-capture detectors. Peaks were identified by their relative retention times obtained on the two columns, using a computer program developed in-house. Quantification will be mainly performed on the Ultra-1 column.

For the 2004 Nunavik Health Survey samples were processed with the ASPE method (Automated Solid Phase Extraction method). This method is based on a fractionation of the plasma extract leading to three fractions (F1, F2, F3), followed by different purification and derivatization methods on F1, F2 and F3, that respectively contain non-polar, non-planar compounds (F1), semi-polar, planar compounds (F2) and polar compounds (F3). Using this sample preparation, PCBs, OC pesticides, and brominated compounds such as BDEs, as well as other compounds can then be measured by mass spectrometry. The final list of compounds measured in the framework of the 2004 Nunavik Health Survey includes 86 analytes: 30 PCB congeners, 16 other OCs (pesticides, phenolic compounds and industrial pollutants) and 26 of their metabolites (15 hydroxy metabolites and 11 methylsulfones), 2 toxaphene congeners (Parlar # 26 and 50) and 12 brominated compounds including PBB-153, TBBA, 4 BDE congeners (congeners # 47, 99, 100 and 153) and brominated metabolites. Limits of detection for the 86 analytes are presented in table 8.

Analysis of PFOS was carried out according to a method recently developed by the INSPQ human toxicology laboratory. This method is based on alkaline extraction with methyl-tert butyl ether and tetrabutylammonium hydrogen-sulfate, followed by electrospray LC-MS-MS analysis. Quantification was carried-out using isotope-labelled internal standards. This analytic method has a detection limit of 0.1µg/L.

Statistical analysis:
Descriptive statistics were performed in order to present plasma concentrations of halogenated hydrocarbons and heavy metals concentrations in whole blood. Student's t-tests were used to assess heavy metals concentrations according to gender, ethnicity (Inuit vs non-native), geographical regions (Hudson Bay vs Ungava Bay) and consumption of marine mammal offals (yes, no) and caribou offals (yes, no). Heavy metals blood levels were also compared using ANOVA test between age categories (18 – 24; 25 – 44; 45 – 74 and 18 – 74 for women of childbearing age), consumption of tobacco (smoker, ex-smoker and non-smoker) and quartiles of marine mammals, fish and game birds consumption. Variation of heavy metal levels between 1992 and 2004, stratified according to gender, age categories, geographical regions and tobacco consumption were compared using Students' t-tests. Blood cadmium concentrations satisfied under the normality criteria and arithmetic mean was therefore used for analysis of variance. Mercury and lead levels were log-transformed in order to approximate a Gaussian distribution and geometric means were used in statistical analysis. A complex study design was developed for the 2004 Nunavik Health Survey to ensure the likelihood that the study population will be representative of the Nunavik Inuit population. A Satterthwaite correction was done on the analysis of variance to account for the study design and only Satterthwaite chi-squared

results and corrected descriptive statistics are presented for heavy metals.

Except for PFOS, data on halogenated hydrocarbons were not available for all participants. Therefore, no Satterthwaite correction was done on descriptive statistics for POPs (except PFOS) and only preliminary, non corrected data are presented for these compounds. We report percent detection, geometric means, 95% confidence intervals and range of values for plasma concentrations of 86 organic contaminants measured in plasma samples from 185 participants. Comparisons of means for PCB congener 153, *p,p'*-DDE and BDE congener 47 between genders and age categories were carried out using Student's t-test and ANOVAs. Comparisons of mean plasma concentrations of organic contaminants between 1992 and 2004 were not carried out because the partial population sample of 2004 presented here is not representative of the Nunavik population (all villages are not represented). These comparisons will be carried out when the complete data set for organic contaminants becomes available.

Results

Descriptive statistics for the blood concentrations of cadmium, mercury and lead detected among Inuit adult population age 18 to 74 years during the 1992 Santé Québec Health Survey and the 2004 Nunavik Health Survey are presented in Table 1. Statistically significant declines ($p < 0.001$) in blood levels for the three heavy metals are observed between 1992 and 2004. Blood lead concentration shows a two-fold decrease over the 12 years period.

Tables 2, 3 and 4 show the 1992 and 2004 mean blood concentrations of heavy metals stratified for gender, age and regions, respectively. Significant decreases in mean heavy metal concentrations have occurred in both genders between the two surveys (Table 2). In 2004, the mean blood mercury levels were significantly higher in women than in men, whereas mean lead levels were higher in men. Cadmium concentrations did not vary significantly according to gender. Heavy metal concentrations have decreased for all age categories between 1992 and 2004, except for cadmium concentrations in adults aged 18-24 years old, who show the highest level (Table 3). Adults aged between 45 and 74 years have significantly higher levels ($p < 0.001$) of mercury and lead than younger adults. As in 1992, non-natives show significantly lower blood concentrations of heavy metals compared to Inuit ($p < 0.001$, data not shown). A significant decrease in heavy metal levels has occurred in communities along the Hudson Bay and Ungava Bay during the 12-years period (Table 4). Mean cadmium and mercury concentrations were significantly higher in communities along the Hudson Bay ($p < 0.001$), but no difference was observed between the two regions for blood lead concentration ($p = 0.187$).

Potential associations between heavy metal blood concentrations, tobacco smoking and traditional food consumption are presented in tables 5, 6 and 7. Since 1992, a significant decrease in cadmium and lead blood levels ($p < 0.01$) has occurred for smokers, ex-smokers and non-smokers (Table 5), with a stronger decrease in cadmium concentrations observed in non-smokers (2-fold) and ex-smokers (3-fold) compared to smokers. Significantly higher concentrations of cadmium were observed in smokers, compared with ex-smokers and non-smokers ($p < 0.001$), whereas no difference in individual lead concentrations appeared according to smoking status ($p < 0,778$). Consumption of marine mammals and caribou offal are not associated with cadmium blood levels in simple regression analysis (data not shown). Mercury concentration increased significantly with quartiles of annual consumption of marine mammals ($p < 0.001$) and fish ($p < 0.001$) (Table 6). Similarly, blood lead level increased moderately with quartiles of game birds annual consumption ($p < 0.001$) (Table 7).

Table 8 shows descriptive statistics for concentrations of organic contaminants measured in plasma of 185 participants from the Inuit Health Survey (2004). A total of 86 analytes were measured, including 29 PCB congeners, 17 chlorinated pesticides, phenols or other chlorinated industrial compounds, 2 toxaphene congeners, 9 brominated compounds including flame retardants and brominated phenols, as well as 11 methylsulfone metabolites of PCBs and DDE and 17 hydroxylated metabolites of PCBs and heptachlorostyrene. Percent detection, geometric means, 95% confidence intervals and range are shown. Means were computed only for analytes detected in more than 50% of samples. For other analytes, we only report percent detection and the range of detected values. For PCBs, a total of 29 congeners were measured, including the traditional suite of PCB congeners measured in earlier studies

Table 1. Average blood levels for heavy metals among Inuit aged between 18 and 74 years for 1992 survey and 2004 survey

	1992					2004							
Contaminant	n	Arithmetic mean	Confidence interval at 95% (lower-upper limit)	Geometric mean	Confidence interval at 95% (lower-upper limit)	n	Arithmetic mean	Confidence interval at 95% (lower-upper limit)	Geometric mean	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	% det.[1]
Cadmium (nmol/L)	492	45.06	(42.52-47.61)	33.20	(30.69-35.90)	917	36.57	(35.05-38.09)	26.57	(25.03-28.20)*	1.4	130.0	100
Mercury (nmol/L)	492	103.75	(96.64-110.90)	74.82	(69.29-80.79)	917	85.96	(79.98-91.93)	51.15	(47.89-54.64)*	0.4	1200.0	100
Lead (µmol/L)	492	0.49	(0.46-0.51)	0.42	(0.40-0.44)	917	0.25	(0.23-0.26)	0.19	(0.18-0.20)*	0.028	2.4	100

[1] % of detection; detection limit (DL): Mercury = 0.5 nmol/L, lead = 0.001 µmol/L, and cadmium = 0.4 nmol/L
* significant variation between health surveys p< 0.001

Table 2. Average blood levels for heavy metals according to gender among Inuit aged between 18 and 74 years for 1992 survey and 2004 survey

		1992			2004					
Contaminant	Gender	n	Mean [1]	Confidence interval at (lower-upper limit)	n	Mean [1]	Confidence interval at (lower-upper limit)	Minimum	Maximum	P value [2]
Cadmium (nmol/L)	Men	252	45.62	-	414	37.02	(35.03-39.01) *	2.10	110.00	0.436
	Women	240	44.48	-	503	36.10	(34.27-37.92) *	1.40	130.00	
Mercury (nmol/L)	Men	252	70.30	(61.45-80.43)	414	45.76	(41.50-50.45) *	0.40	1200.00	< 0.001
	Women	240	79.86	(73.51-86.77)	503	57.58	(53.66-61.79) *	1.00	820.00	
Lead (µmol/L)	Men	252	0.46	(0.43-0.50)	414	0.22	(0.21-0.24) *	0.044	2.40	< 0.001
	Women	240	0.38	(0.35-0.40)	503	0.17	(0.16-0.17) *	0.028	1.50	

[1] Arithmetic mean for cadmium and geometric mean for lead and mercury
[2] variation between gender for 2004; associated with the Satterthwaite χ^2
* significant variation between health surveys p< 0.001
- not available

Table 3. Average blood levels for heavy metals according to age group among Inuit aged between 18 and 74 years for 1992 survey and 2004 survey

		1992			2004					
Contaminant	Age group	n	Mean [1]	Confidence interval at 95% (lower-upper limit)	n	Mean [1]	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	P value [2]
Heavy metals										
Cadmium (nmol/L)	18 to 24 years	137	43.85	(38.84-48.86)	206	45.43	(42.34-48.52)	2.10	110.00	0.004
	25 to 44 years	235	48.08	(45.87-55.89)	471	37.68	(35.48-39.89) *	1.40	110.00	
	45 to 74 years	121	40.56	(38.01-48.77)	240	26.15	(23.45-28.85) *	2.00	130.00	
	Women of childbearing age (18 to 39 years)	175	46.66	(42.85-50.48)	308	38.13	(36.01-40.26)*	1.40	110.00	
Mercury (nmol/L)	18 to 24 years	137	50.64	(43.80-58.55)	206	31.45	(27.65-35.77) *	2.20	820.00	< 0.001
	25 to 44 years	235	69.15	(62.24-76.83)	471	44.31	(40.12-48.94) *	0.40	420.00	
	45 to 74 years	120	135.91	(120.49-153.29)	240	106.57	(96.08-118.2)**	4.60	1200.00	
	Women of childbearing age (18 to 39 years)	175	64.51	(59.18-70.30)	308	41.73	(38.16-45.63)*	1.00	820.00	
Lead (µmol/L)	18 to 24 years	137	0.31	(0.28-0.35)	206	0.14	(0.13-0.15)*	0.033	0.80	< 0.001
	25 to 44 years	235	0.43	(0.40-0.46)	471	0.19	(0.17-0.20)*	0.028	2.40	
	45 to 74 years	121	0.56	(0.52-0.61)	240	0.29	(0.27-0.31)*	0.039	1.50	
	Women of childbearing age (18 to 39 years)	175	0.33	(0.31-0.36)	308	0.13	(0.12-0.14)*	0.028	1.00	

[1] Arithmetic mean for cadmium and geometric mean for lead and mercury
[2] variation with age for 2004; associated with the Satterthwaite χ^2
*significant variation between health surveys p< 0.001
**significant variation between health surveys p< 0.01

Table 4. Average blood levels for heavy metals according to coastal region among Inuit aged between 18 and 74 years for 1992 survey and 2004 survey

		1992			2004					
Contaminant	Coastal region	n	Mean [1]	Confidence interval at 95% (lower-upper limit)	n	Mean [1]	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	**P value** [2]
Cadmium (nmol/L)	Hudson	274	48.42	(44.85-52.00)	497	39.46	(37.38-41.53) *	2.00	110.00	< 0.001
	Ungava	219	40.22	(36.66-43.77)	420	32.80	(30.50-35.10)*	1.40	130.00	
Mercury (nmol/L)	Hudson	291	93.04	(84.74-102.15)	497	58.65	(53.12-64.76) *	0.40	1200.00	< 0.001
	Ungava	201	54.60	(48.56-61.39)	420	42.77	(38.89-47.04)*	1.00	520.00	
Lead (µmol/L)	Hudson	291	0.48	(0.45-0.51)	497	0.20	(0.19-0.21)*	0.036	2.40	0.187
	Ungava	201	0.35	(0.32-0.38)	420	0.19	(0.18-0.20)**	0.028	1.40	

[1] Arithmetic mean for cadmium and geometric mean for lead and mercury
[2] Variation between coastal region for 2004; associated with the Satterthwaite χ^2
* significant variation between health surveys p< 0.001
** significant variation between health surveys p< 0.01

Table 5. Average blood levels for cadmium and lead according to tobacco use among Inuit aged between 18 and 74 years for 1992 survey and 2004 survey

		1992			2004					
Contaminant	Smoker status	n	Mean [1]	Confidence interval at 95% (lower-upper limit)	n	Mean [1]	Confidence interval at 95% lower-upper limit)	Minimum	Maximum	P value [2]
Heavy metals										
Cadmium (nmol/L)	Smoker	292	58.03	-	663	45.07	(43.45-46.69)*	3.20	130.00	< 0.001
	Ex-smoker	91	21.64	-	119	7.63	(6.83-8.42)*	2.00	23.00	
	Non-smoker	49	11.08	-	76	5.91	(5.17-6.65)**	1.40	22.00	
Lead (µmol/L)	Smoker	292	0.43	(0.41-0.46)	663	0.19	(0.18-0.20)*	0.028	1.40	0.778
	Ex-smoker	91	0.40	(0.36-0.44)	119	0.19	(0.17-0.21)*	0.044	2.40	
	Non-smoker	49	0.33	(0.28-0.39)	76	0.18	(0.16-0.21)*	0.033	1.00	

[1] Arithmetic mean for cadmium and geometric mean for lead and mercury
[2] variation with smoker status for 2004; associated with the Satterthwaite χ^2
*significant variation between health surveys p< 0.001
** significant variation between health surveys p< 0.01
- not available

Table 6. Average blood levels for mercury according to quartiles of consumption of marine mammals meat and country fish (gr/day, annual) among Inuit aged between 18 and 74 years (2004 survey)

		Mercury (nmol/L)					
		n	Geometric mean	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	P value[1]
Consumption of marine mammals (Quartiles)	Low (0-25%)	179	28.04	(23.77-33.09)	0.97	520.00	< 0.001
	Low-Moderate (25-50%)	204	44.61	(39.08-50.93)	1.80	460.00	
	High-Moderate (50-75%)	193	66.54	(57.95-76.41)	3.20	1200.00	
	High (75-100%)	192	80.13	(70.91-90.54)	3.00	760.00	
Consumption of country fish (Quartiles)	Low (0-25%)	193	36.70	(31.06-43.37)	1.00	820.00	< 0.001
	Low-Moderate (25-50%)	192	48.97	(42.31-56.68)	0.97	760.00	
	High-Moderate (50-75%)	192	56.76	(48.04-67.05)	1.10	1200.00	
	High (75-100%)	194	69.25	(60.70-79.00)	5.10	720.00	

[1] associated with the Satterthwaite χ^2

(IUPAC # 99, 101, 118, 128, 138, 153, 156, 170, 180, 183, 187). The most abundant congeners are IUPAC # 153, 180 and 138. Among those measured, six congeners are mono-ortho substituted PCBs (IUPAC# 105, 118, 156, 167, 189) and their levels are generally lower than levels observed for poly-ortho substituted congeners. Most congeners were detected in more than 50% of samples, except for IUPAC # 118, 128 and 189.

In the category of the chlorinated pesticides and other industrial compounds, *p,p'*-DDE, trans-nonachlor, pentachlorophenol and oxychlordane show the highest levels. Other compounds previously measured in the traditional suite of POPs were also measured (cis-Nonachlor, HCB, Mirex, *p,p'*-DDT, α-HCH).

Additional compounds were included in the suite of POPs measured. These included two congeners of toxaphene, brominated compounds, methylsulfone metabolites and hydroxylated metabolites. Toxaphenes were detected in more than 90% of samples, with levels ranging from 6 to 5913 ng/L. Among the brominated compounds, PBDEs # 47, 99, 100 and 153 were measured, but only # 47 and 153 were detected in more than 50% of samples, with levels ranging from 30 to 2400 ng/L and 10 to 406 ng/L, respectively. PBB # 153, pentabromophenol, tetrabromobisphenol A as well as two tetrabromophenols were measured, but only 2,3,4,6-TBP was detected in more than 50% of samples with levels ranging from 8 to 1395 ng/L.

Nine methylsulfone metabolites of PCBs and DDE were measured, and five of them were detected in more than 50% of samples. Most abundant ones were 3-Methylsulfonyl-PCB-49 and 3-Methylsulfonyl PCB-101. Seventeen hydroxylated metabolites of PCBs and heptachlorostyrene were measured and all of them except 2-hydroxy-PCB-28 were detected in more than 50% of samples. The most abundant metabolites are 4-OH-PCB-107, 4-OH-PCB-146, 4-OH-PCB-187, 3-OH PCB-153 and 3-OH-PCB-138.

In addition to these organic contaminant, PFOS was measured and results are available for the complete population sample (917 participants) of the Inuit Health Survey (2004). The mean concentration (geometric) was 18.38 µg/L ($CI_{95\%}$[17.67 -19.13] (corrected with Satterthwaite procedure).

Concentrations of PCB-153, PBDE-47 and *p,p'*-DDE stratified by age groups and by gender are presented in Tables 9 and 10, respectively. Groups were compared by ANOVA. This represents only a preliminary analysis on the 185 samples for which data was available. There was a significant increase in concentrations of PCB-153 and *p,p'*-DDE in relation to age groups, but PBDE 47 did not show an age-related increase or decrease. PCB-153 and *p,p'*-DDE were highest in people aged 45 to 74 years. PBDE-47 showed slightly higher levels in women aged 18 to 39 years, but this difference with other age groups was not statistically significant. In table 10, the concentrations of PCB-153, *p,p'*-DDE and

Table 7. Average blood levels for lead according to quartiles of consumption of game birds (gr/day, annual) among Inuit aged between 18 and 74 years (2004 survey)

		Lead (µmol/l)					
		n	Geometric mean	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	P value[1]
Consumption of game birds (Quartiles)	Low (0-25%)	188	0.16	(0.14-0.18)	0.033	2.40	< 0.001
	Low-Moderate (25-50%)	192	0.19	(0.18-0.21)	0.032	1.40	
	High-Moderate (50-75%)	195	0.21	(0.20-0.23)	0.040	1.40	
	High (75-100%)	194	0.21	(0.19-0.24)	0.046	1.20	

[1] associated with the Satterthwaite χ^2

PBDE-47 are stratified by gender. For all three compounds, there are no significant differences in concentrations measured in women and men, although ranges of values observed are larger in women than in men.

Discussion and Conclusion

This report presents data on environmental contaminants exposure obtained from the Inuit Health Survey carried out in Fall 2004. At the time of writing this report, data was available on the complete population sample for heavy metals and PFOS, but other POPs were measured only on a sub-sample of 185 participants, which were not evenly distributed among villages. Here we report on the available data and comparisons with the 1992 Santé Québec survey are carried-out only on heavy metals.

Statistically significant declines in blood concentrations of heavy metals have occurred between 1992 and 2004. Blood lead concentration shows a two folds decrease over the 12 years period. Levels of lead are now within the reference values (0.04-0.32 µmol/l) for the general population of Quebec (Leblanc, et al. 2004). Mean lead levels are also lower than the tolerable daily intake level recommended by the World Health Organisation of 3.57 µg/kg/day (also followed by Health Canada), which is equivalent to 0.48 µmol/L (Van Oostdam, et al. 2005; WHO. 2000). However, some individuals are still over this level, with a maximum observed of 2,4 µmol/L. Appropriate follow-up is undertaken for these individuals. This strong decrease in lead levels may be due to the ban on lead shots used for hunting, implemented by the Public Health Directorate in 1998 (Levesque, et al. 2003). The public health authorities of Nunavik also actively informed the population about the toxic effect of lead from ammunition on children health in order to reduce exposure (Levesque, et al. 2003).

Evidence for increasing levels of mercury in the Canadian Arctic is observed in a number of marine birds and mammals (AMAP. 2002a). However, human blood levels do not follow this increasing trend and show a decrease of 30%, which could be attributable to changes in dietary habits. The latter could result from the promotion of less contaminated food (like Artic char) in the Arctic (Berti, et al. 1998) or from the decrease in traditional food consumption (and consequent shift to a non-traditional, westernized diet). The 1992 survey supports this and showed that the dietary habits of the Inuit population are changing and that consumption of traditional food, like fish and marine mammal, is decreasing (Dewailly, et al. 1994). However, despite the observed decrease in blood mercury levels, the latter remain higher then the reference levels for the population of Quebec (reference levels of <0.1-16 nmol/L)(Leblanc, et al. 2004), but they are lower than the tolerable daily intake level established by the World Health Organisation of 0.23 µg/kg/day, which is equivalent to 67 nmol/L (WHO. 2000). Nevertheless, the range of observed levels reaches a maximum of 1200 nmol/L, showing that some individuals are well over the recommended level. Appropriate follow-up for these individuals is ongoing.

In 2004, average blood mercury level was higher in women than in men, whereas average lead level was higher in men. Associations between mercury and gender observed in other studies are inconsistent and

Table 8. Plasma concentrations of several organic contaminants (ng/L). Preliminary data obtained from 185 participants from the Inuit Health Survey (2004)

Contaminant	n	Detection limit (ng/L)	% Detected	Geometric Mean (ng/L)	GM CI $_{95\%}$	Minimum	Maximum
PCB (IUPAC #)							
Aroclor 1260	169		100	9769.51	(7909.84-12066.4)	410	310000
74	169	4	94	69.16	(57.29-83.51)	11	2600
99	169	5	98	170.5	(140.93-206.27)	12	4700
101	169	2	65	32.24	(27.43-37.89)	10	470
105	169	2	74	45.7	(37.55-55.61)	10	1200
118	169	2	9			10	5300
128	169	2	34			10	200
138	169	2	100	533.61	(435.52-653.8)	27	19000
146	169	1	97	180.5	(146.93-221.75)	12	6100
153	169	2	100	1332.98	(1074.56-1653.55)	48	40000
156	69	2	88	89.97	(73.28-110.45)	10	2400
157	169	2	65	44.77	(37.19-53.89)	10	610
163	169	3	98	220.82	(178.39-273.34)	12	6200
167	169	1	66	35.39	(29.2-42.89)	10	880
170	169	2	99	216.16	(173.33-269.57)	12	6182
172	169	4	78	71.73	(58.71-87.63)	11	1400
177	169	2	83	52.6	(44.14-62.7)	10	1700
178	169	1	91	86.99	(71-106.59)	11	1900
180	169	2	100	813.3	(645.99-1023.95)	24	22727
183	169	1	94	86.95	(71.97-105.05)	10	2800
187	169	1	100	286.8	(232.88-353.19)	11	9100
189	169	3	49			10	270
194	169	3	95	182.08	(143.88-230.43)	10	5100
195	169	2	63	45.99	(38.38-55.1)	10	760
196	169	4	76	53.52	(44.27-64.71)	10	1500
201	169	4	97	167.21	(132.53-210.97)	11	4700
203	169	2	92	105.1	(85.03-129.91)	10	2500
206	169	3	85	80.16	(64.23-100.04)	10	1700
208	169	2	64	59.76	(47.95-74.48)	10	880
209	169	3	59	53.32	(43.04-66.07)	9	619
Chlorinated pesticides and other industrial compounds							
α-HCH	169	10	9			10	48
cis-Nonachlor	169	2	98	108.29	(88.91-131.89)	6,5	3500
Hexachlorobenzene	169	16	98	290.96	(241.75-350.19)	21	7400
Kepone	168	38	57	268.96	(219.13-330.12)	55	4400
Mirex	169	3	93	110.65	(88.61-138.17)	9	3636
Oxychlordane	169	2	100	430.83	(342.81-541.46)	12	16000
Octachlorostyrene	169	2	30			5,1	41
p,p'-DDD	169	31	1			71	100
p,p'-DDE	169	15	100	3231.8	(2734.24-3819.89)	300	50000
p,p'-DDT	169	7	94	87.69	(76.21-100.89)	20	1600
ß-HCH	169	5	91	50.95	(43.23-60.05)	10	1200
trans-Nonachlor	169	1	100	724.83	(585.55-897.25)	15	22000
2,3,4,6-Tétrachlorophénol	150		51	27.07	(23.13-31.68)	10	133
Pentachloroanisole	169	4	7			10	14
Pentachlorobenzène	169	5	28			10	40
Pentachloronitrobenzène	169	5	1			11	21
Pentachlorophénol	150	10	100	914.17	(800.16-1044.43)	190	18000

Table 8. Continued

Table 8. Plasma concentrations of several organic contaminants (ng/L). Preliminary data obtained from 185 participants from the Inuit Health Survey (2004)

Contaminant	n	Detection limit (ng/L)	% Detected	Geometric Mean (ng/L)	GM CI $_{95\%}$	Minimum	Maximum
Toxaphene							
Parlar # 26	169	3	93	92.03	(75.06-112.82)	8	3391
Parlar # 50	169	1	99	142.41	(115.77-175.19)	6	5913
Brominated flame retardants and other compounds							
PBB IUPAC # 153	166	4	45			10	138
PBDE IUPAC # 47	166	7	55	72.48	(60.26-87.17)	30	2400
PBDE IUPAC # 99	160	7	23			18	575
PBDE IUPAC # 100	166	3	19			21	580
PBDE IUPAC # 153	166	4	67	22.29	(19.61-25.33)	10	406
PBP	150		22			2,1	45
Tétrabromobisphénol-A	150	20	17			21	200
2,3,4,5-Tétrabromophénol	150	10	8			2,1	41
2,3,4,6-Tétrabromophénol	150	2	63	35.94	(30.57-42.26)	8	1395
Methylsulfone metabolites							
3-Methylsulfonyl-PCB 49	164	2	96	31.32	(25.77-38.06)	2,4	910
3-Methylsulfonyl-PCB 87	164	5	79	16.26	(13.38-19.75)	2,1	440
3-Methylsulfonyl-PCB 101	164	2	86	29.71	(24.22-36.44)	2,3	920
3-Methylsulfonyl-PCB 141	164	5	33			5	130
3-Methylsulfonyl-PCB 149	164	5	21			5,3	45
3-Methylsulfonyl-DDE	164	5	49			5,1	470
4-Methylsulfonyl-PCB 49	164	5	70	12.34	(10.01-15.21)	2	220
4-Methylsulfonyl-PCB 87	164	5	52	21.8	(17.94-26.47)	5,1	310
4-Methylsulfonyl-PCB 91	164	5	18			2	17
4-Methylsulfonyl-PCB 101	164	5	74	13.02	(10.55-16.07)	2	250
4-Methylsulfonyl-PCB 149	164	5	31			5,1	88
Hydroxylated metabolites							
2-Hydroxy-PCB 68	150	10	28			10	120
2-Hydroxy-PCB 75	150	5	52	16.28	(13.82-19.19)	5,9	120
3-Hydroxy-PCB 138	149	2	90	35.12	(28.05-43.98)	2	750
3-Hydroxy-PCB 153	150	2	96	48,.6	(38.66-62)	2,2	1500
3-Hydroxy-PCB 180	151	2	68	8.2	(6.61-10.16)	2	303
4-Hydroxy-PCB 107	149	10	99	150.95	(127.55-178.64)	17	1333
4-Hydroxy-PCB 146	150	2	98	151.08	(121.34-188.1)	2,7	2400
4-Hydroxy-PCB 163	150	2	88	13.49	(11.1-16.39)	2	180
4-Hydroxy-PCB 172	149	2	97	20.74	(16.84-25.55)	2	450
4-Hydroxy-PCB 187	150	2	99	132.37	(110.02-159.25)	7,1	1600
4-Hydroxy-PCB 193	150	2	58	6.45	(5.28-7.87)	2	83
4-Hydroxy-PCB 199	151	2	93	26.41	(21.36-32.65)	2	1200
4-Hydroxy-PCB 200+198	150	4	55	9.89	(8.05-12.16)	2	93
4-Hydroxy-PCB 201	150	2	50	4.3	(3.7-4.99)	2	21
4-Hydroxy-PCB 202	150	2	91	9.24	(7.64-11.19)	1	288
4-Hydroxy-PCB 208	151	2	77	7.31	(6.06-8.83)	2	226
4-Hydroxy-Heptachlorostyrene	150	1	100	30.37	(25.01-36.89)	1	485

Table 9. Plasma concentrations of PCB-153, PBDE-47 and *p,p'*-DDE stratified for age groups (ng/L). Preliminary data obtained from 185 participants

Contaminant	Age group	n	% detected	Geometric mean (ng/L)	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	P value [1]
PCB 153	18 to 24 years	42	100	435	(314-604)	48	2200	< 0.0001
	25 to 44 years	81	100	828	(657-1044)	53	8200	
	45 to 74 years	62	100	4600	(3393-6236)	67	40000	
	Women of childbearing age (18 to 39 years)	60	100	463	(357-602)	48	3900	
PBDE 47	18 to 24 years	42	56	60.6	(42.8-85.8)	30	520	0.6166
	25 to 44 years	81	58	75.4	(55.7-102.2)	30	2400	
	45 to 74 years	62	50	76.7	(55.6-105.9)	30	1300	
	Women of childbearing age (18 to 39 years)	60	60	80.1	(54.1-118.6)	30	2400	
p,p'-DDE	18 to 24 years	42	100	1391	(1138-1701)	380	4400	< 0.0001
	25 to 44 years	81	100	2145	(1784-2580)	300	14000	
	45 to 74 years	62	100	8754	(6921-11073)	470	50000	
	Women of childbearing age (18 to 39 years)	60	100	1574	(1285-1929)	300	7200	

[1] ANOVA using the three age categories. Women of child bearing age are not included as an age category in the analysis.

vary in different populations (Dewailly, et al. 2001; Dumont, et al. 1998; Kosatsky, et al. 2000), but higher levels of lead in men have already been reported in other studies (Bjerregaard, et al. 2004; Chu, et al. 1999; Ducoffre, et al. 1990). However, a clearer association between age and lead/mercury blood levels is observed. Adults aged between 45 and 74 years had significant higher level ($p < 0.001$) of mercury and lead than younger adult, as seen in other studies (Bjerregaard and Hansen. 2000; Bjerregaard, et al. 2004; Dewailly, et al. 2001; Rhainds, et al. 1999). This increasing trend in relation to age probably reflects higher intake of traditional food. As in other studies, mercury blood concentrations increased significantly with quartiles of annual consumption of mammals (Bjerregaard and Hansen. 2000; Dewailly, et al. 2001; Grandjean, et al. 1992) and fish (Cole, et al. 2004; Grandjean, et al. 1992; Mahaffey and Mergler. 1998)($p < 0.001$) (Table 6). Similarly, blood lead level increased moderately with quartiles of annual game birds consumption ($p < 0.001$), which is consistent with other studies (Bjerregaard, et al. 2004; Dewailly, et al. 2001; Hanning, et al. 2003; Johansen, et al. 2006). However, many studies have also shown an association between blood lead levels and smoking (Dewailly, et al. 2001; Grandjean, et al. 1992; Levesque, et al. 2003; Rhainds, et al. 1999), but this association could not be observed in the current study, hence suggesting that smoking does not represent a significant source of lead exposure in the Nunavik population and that dietary intake represents the main source of exposure.

Further analyses of dietary intake of lead and mercury are needed in order to determine if a general decrease in the intake of traditional food explains the decrease in these heavy metals exposure from 1992 to 2004, or if the decrease is attributable to a shift from more to less contaminated traditional food intake. Associations with gender and age groups

Table 10. Plasma concentrations of PCB-153, PBDE-47 and *p,p'*-DDE stratified for gender (ng/L). Preliminary data obtained from 185 participants

Contaminant	Gender	n	% detected	Geometric mean	Confidence interval at 95% (lower-upper limit)	Minimum	Maximum	P value [2]
PCB 153	men	80	100	1471	(1078-2005)	53	14000	0.4325
	women	105	100	1236	(915-1671)	48	40000	
PBDE 47	men	80	52	61.6	(50.2-75.8)	30	400	0.1507
	women	105	57	81.0	(61.3-107.0)	30	2400	
p,p'-DDE	men	80	100	3005	(2390-3778)	310	19000	0.4560
	women	105	100	3415	(2685-4342)	300	50000	

[2] ANOVA using the gender categories.

could also be explained by differences in traditional food consumption and the ongoing analyses of dietary questionnaires from the Inuit Health Survey aim at providing answers to these questions.

Blood cadmium levels showed a 22% decrease between 1992 and 2004. Concentrations did not vary according to gender, like it was observed in other studies (Benedetti, et al. 1999; Rey, et al. 1997). Decreases were observed in age groups above 25 years old, but cadmium concentrations in adults aged 18-24 years old did not change from 1992 to 2004 and this group showed the highest levels. Levels of blood cadmium observed in Nunavik are nevertheless within the reference values for the general population of Québec (1.8-55 nmol/L) (Leblanc, et al. 2004). Both WHO and Health Canada recommend a tolerable daily intake level of 1 µg/kg/day, which is equivalent to 44.5 nmol/L (WHO. 2004). The mean cadmium levels observed are below this value, but as for mercury and lead, some individual remain above this level (maximum of 130 nmol/L). It is unlikely that decreases in blood Cd be attributable to dietary shifts, since smoking was found to be the main source of cadmium exposure in Nunavik (Benedetti, et al. 1994; Rey, et al. 1997) and the association of cadmium with smoking status has been observed in numerous other studies (Benedetti, et al. 1992; Butler Walker, et al. 2006; Levesque, et al. 2003). In support to this, we observed that consumption of marine mammals and caribou offals were not associated with cadmium blood levels in simple regression analysis (data not shown). The decrease in Cd blood levels could be partially explained by a decrease in the cadmium content of cigarettes sold in Canada. Indeed, blood cadmium in Québec City smokers was 46 nmol/L in 1994 (Benedetti, et al. 1994) and 10 nmol/L in 2001 (Leblanc, et al. 2004), which represents a decrease of 80%. A sound evaluation of smoking habits considering gender and age differences, the age when people started smoking, numbers of cigarette smoked per day, cigarette brands and exposure to second-hand smoke, would be needed to better explain changes (or absence of changes) in blood levels of cadmium. As far as we know, the smoking rate did not decrease significantly in Nunavik and this public health issue would deserve more attention, especially in younger age groups.

Exposure to persistent organic pollutants could not be thoroughly interpreted in the current report given the partial results reported. The partial results obtained in 2004 show that the mean (geometric) level of Aroclor 1260 is of 9,.7 µg/L of plasma (corresponding approximately to 4,.8 µg/L whole blood), which is just below the level of concern determined by Health Canada for maternal blood (5 µg/L blood) (Van Oostdam, et al. 1999). Some individuals are above this level, but the maximum concentration observed is 31 µg/L (corresponding approximately to 15,.5 µg/L whole blood), which is well below the level of action recommended by Health Canada.

A decrease in organochlorine exposure is observed in adults aged between 18 and 74 years since 1992. The mean (geometric) level of Aroclor 1260 computed for the 185 participants of the 2004 study is 9,.77 µg/L with a $CI_{95\%}$ of [7,91-12,07], while the

mean (geometric) level reported in the Santé Québec study for 492 participants was 16,.12 µg/L ($CI_{95\%}$[14,67-17,71]). For other organochlorines, 1.5 to 3-fold decreases are observed. These preliminary data are consistent with previous observations reported in temporal trend studies addressing organochlorine exposure in the Nunavik population (Dallaire, et al. 2003b; Pereg, et al. 2003) and in other populations (Dallaire, et al. 2002b; LaKind, et al. 2001; Meironyte, et al. 1999). It is likely that these decreases in plasma concentrations are attributable to changes in diet and to slight decreases of certain POPs in the environment. However, further analyses on PCB congener profiles, as well as comparisons with accurate concentrations computed on the complete population sample of 2004 remain to be finalized. Additionally, analyses on dietary intake of organochlorines will allow the identification of sources of exposure and will provide explanations for the decrease observed.

Despite this decrease, levels of exposure to PCBs are still close to the level of concern and chronic exposure to PCBs and other organochlorines have been related to adverse effects on development (Muckle et al, in preparation) and on immune function (Dallaire, et al. 2004; Dallaire, et al. 2006; Dewailly, et al. 2000) in Nunavik children. Therefore, monitoring of these contaminants should continue and information on sources of exposure should be provided to the population in order to attempt to reduce exposure down to background levels.

Toxaphene Parlar # 26 and 50 were also measured in this study. Levels observed for Parlar # 26 and # 50 (geometric mean and $CI_{95\%}$: 0,.092 µg/L [0,.075-0,.112] and 0,.142 [0,.115-0,.175], respectively) were similar than those reported in Inuit from the Baffin and Kivallik area in a study addressing maternal and umbilical cord blood in Arctic Canada (Butler Walker, et al. 2003). We could not compare the current levels of toxaphenes with those prevailing in 1992 because these compounds were not measured then. Efforts will be made in other projects to document temporal changes in toxaphene exposure.

In this report, we also present data for emerging compounds such as brominated flame retardants and metabolites of PCBs and *p,p'*-DDE. Since several brominated POPs exert toxic effects that are similar to those exerted by PCBs, dioxins-like chemicals and organochlorine pesticides (Branchi, et al. 2003; Darnerud, et al. 2001; Zhou, et al. 2002), and since their levels in the environment (Ikonomou, et al. 2002) as well as in human tissues and fluids (Meironyte, et al. 1999; Noren and Meironyte. 2000; Pereg, et al. 2003) have been reported to rise dramatically since the past decade, monitoring exposure to these contaminants and investigating sources of exposure is of utmost importance to avoid undue exposures, despite the fact that guidelines regarding tolerable daily intake and levels of concerns are still at the experimental level (Gill, et al. 2004). Additionally, more experimental and epidemiological studies are needed to assess the toxic effects exerted by methylsulfone and hydroxylated metabolites of PCBs and to determine levels of concern for human plasma levels of metabolites. The levels reported here for these emerging compounds will serve as a baseline for further studies.

When data for PCB-153, *p,p'*-DDE and PBDE-47 are stratified for gender and age groups, we observe no effect of gender on plasma concentrations of these compounds. However, a significant effect of age groups is noted on plasma concentrations of organochlorines, which increase with age, whereas this association is not observed for PBDEs. This observation most likely reflects the higher intake of PCBs and *p,p'*-DDE through dietary routes, as well as the bio-accumulation of these compounds. PBDEs are also prone to bioaccumulation, but it is likely that exposure to these compounds is more ubiquitously distributed among age groups and that exposure to the latter is more recent.

Further analyses will complete the exposure assessment component regarding POPs. Many questions and hypotheses are raised regarding the sources of exposure, the influence of diet and the possible toxic effects of emerging contaminants. Further analyses of the data collected during the Qanuippitaa survey will help provide answers to some of these questions. These analyses are ongoing and results should be communicated as soon as they are available.

Expected Project Completion Date

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