Chapter 6

Toxicological Properties of Persistent Organic Pollutants and Related Health Effects of Concern for the Arctic Populations

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Summary

Human exposure to environmental contaminants is ubiquitous and not only limited to individuals living close to the sources of contaminants. Everyone carries a burden of persistent organic pollutants (POPs) in their body. The burden of POPs in Arctic peoples has been monitored for some years, however, it is only recently that a programme for measuring the potential biological effects of these contaminants has been established: the AMAP Human Health Effects Monitoring Programme. Body burden data alone are not enough to allow the health risks associated with exposure to environmental contaminants in Arctic peoples to be assessed. Furthermore, laboratory studies on the effects of single chemicals or chemical mixtures in laboratory animals and cell cultures cannot fully elucidate the human health risks. Integration of epidemiological and biomarker studies on humans from exposed populations in the Arctic is needed in order to obtain information about the real health risks resulting from exposure to the accumulated mixtures of contaminants in the Arctic.

The broad category of human health effects that are suspected to result from exposure to environmental contaminants include cancer, birth defects, effects on the reproductive and the neuro-endocrine-immune systems, altered metabolism, and specific organ dysfunction. This chapter gives an introduction to these various health effects and presents possible biomarkers that may be useful to include in epidemiological studies. It also discusses the connection between traditional toxicological studies and new methods designed to study the potential of chemicals to interfere with the normal homeostasis by exerting endocrine-disrupting effects.

6.1. Overview

The AMAP Phase I assessment report (AMAP, 1998) included an overview of the classical toxicology of contaminants. In 1997, the Alta Declaration extended AMAP's mandate to cover assessment of the combined effects of environmental stressors. This chapter presents a comprehensive and detailed description of the rationale for conducting effects studies. There is some overlap with that part of chapter 9 dealing with epidemiological studies, but this is necessary to discuss the evaluation of effects parameters. Several studies considered below were conducted outside the Arctic as only a few investigations have been carried out in this region to date.

Over the past decade most efforts have been focused on the characterization of exposure of Arctic peoples to contaminants. Epidemiological studies have been conducted in which clinical endpoints, such as psychometrics, neurophysiological parameters, infection incidence, bone density, and sexual maturation among others, were the main focus. However, in order to detect the early biological changes preceding disease, knowledge about the mechanism of action of toxicants is required. Thus, biomarkers of effect need to be validated and used. Effect biomarkers are early biological responses of the organism to an external toxic stress. Since the overall weight of evidence at the epidemiological level for adverse endocrine-related human health effects is not strong, further studies including validated biomarkers in epidemiological studies may help in identifying the possible relevant associations between exposure to contaminants and detrimental health effects in Arctic populations.

The Canadian Arctic Contaminants Assessment Report (Jensen *et al.*, 1997) identified areas with information gaps toward which Arctic contaminant research programmes should be oriented; effect biomarkers were, however, not considered. They were, however, adopted for use in the AMAP Phase II Human Health Effects Monitoring Programme (see section 6.2).

In this chapter, the main focus is on effect biomarkers of early biological responses to evaluate neurobehavioral, immunological, and reproductive organ status of newborns in the Arctic. It therefore, concentrates on biological effects related to reproductive and developmental effects, the neurological and immune system function, and oxidative stress. Discussions are mainly restricted to cover effects due to POPs, including methylmercury (MeHg). Other contaminants, such as cadmium (Cd) and lead (Pb), are now considered of lower priority. Their major sources, relevant to human health in the Arctic, are smoking (for Cd) and lead shot (for Pb) and information on their toxicity and on biomarkers of effects is already available, validated and widely used (e.g., urinary β2-microglobulin level, blood δ-aminolevulinic dehydratase activity). Furthermore, benchmark doses as well as biological guidelines for Cd and Pb have been adopted by international health organizations. In contrast, health risk assessments related to the presence of MeHg and POPs in the Arctic food chain carry much more uncertainty.

6.1.1. The emergence of endocrine disruption

Human exposure to environmental contaminants is ubiquitous. Exposure is not restricted to individuals who live next to industries or waste disposal sites, or those who reside in inner cities or third-world countries

where, e.g., insecticides are widely used. Everyone carries a burden of POPs and heavy metals in their body. Persistent organochlorine (OC) compounds, such as dioxins/furans, polychlorinated biphenyls (PCBs) and certain pesticides, e.g., toxaphene and DDT/DDE, accumulate in body fat; Hg accumulates in organs, Pb in bones, etc. Environmental contamination is a global issue and POPs are transported to the Arctic by atmospheric and oceanic currents. Because of the lipophilic and persistent nature of POPs, bioaccumulation and biomagnification occur in the Arctic marine food web and in some freshwater predatory fish and piscivorous birds. Although far distant from the major pollution sources, some populations living in regions north of the Arctic Circle display a greater body burden of POPs than people living in industrialized regions, largely due to their reliance on a traditional diet that includes species high up in the marine food chain (Asplund *et al.*, 1994; Dewailly *et al.*, 1992, 1994b, 1999; Jensen and Clausen, 1979). Use of several OCs, such as DDTs and PCBs, was restricted or banned in most countries in the 1970s. Even though their concentrations in the environment have been slowly declining over the past 30 years, these compounds are still the most abundant persistent OCs found in wildlife and in human tissue and milk samples in the Arctic region (Safe, 2000).

While concentrations of several OCs are decreasing, the continual introduction of 'new compounds', such as brominated flame retardants, into the environment has generated new concerns (Rahman *et al.*, 2001). In addition, attention has recently shifted from chronic diseases and reproductive endpoints to effects that are induced following exposure during the sensitive period of *in utero* development. Of particular concern are effects on development resulting from prenatal exposure to endocrine-disrupting compounds.

To date, no clear-cut evidence for adverse endocrine-related human health effects has been obtained at the individual or population level. However, data from studies on wildlife species, studies on laboratory animals, and biomarker studies *in vitro* have strengthened the need for further research to address the uncertainty and alleviate concerns. Taking a precautionary approach, the weight of evidence would suggest that exposure levels seen in the Arctic have some potential for adverse effects on human health.

Studies on wildlife populations have documented adverse effects that correlate with exposure to one or more putative endocrine-modulating chemicals (Safe, 2000). Adverse developmental and reproductive effects have been primarily linked to POPs and alkylphenols derived from alkylphenol ethoxylate surfactants used in industrial detergents. In many instances, it has been difficult to assign causality because of the complexity of environmental contaminant mixtures and the level of exposure during critical developmental windows. However, lower concentrations of POPs in the Great Lakes region were correlated with dramatic improvements in reproductive success and significant increases in an array of predatory birds in the Great Lakes basin (Tremblay and Gilman, 1995).

The range of toxicological effects that estrogenic chemicals can produce is illustrated by work on the synthetic estrogen diethylstilbestrol (DES). DES was used pharmaceutically from the late 1940s to the early 1970s to prevent miscarriages and pregnancy complications in women. However, it was removed from the market in the 1970s when studies found DES exposure to correlate with increases in abortions, neonatal death and premature birth, and an increase in the incidence of vaginal adenocarcinoma in young women who were exposed *in utero* (Herbst *et al.*, 1971). A study of men exposed *in utero* showed 31.5% had abnormalities of the reproductive tract compared with 7.8% of controls (Gill *et al.*, 1979). The abnormalities included cryptorchidism and hypospadias, and reduced sperm concentration and quality, although reduced fertility was not observed in these men (Wilcox *et al.*, 1995). Exposure of mice *in utero* induced very similar effects to those seen in humans (McLachlan, 1981). Not all the effects of DES are ascribed to its binding to the estrogen receptor and recent studies have shown that several endocrine-disrupting compounds induce their effects via different receptors and signaling pathways (Andersen *et al.*, 2002; Bonefeld-Jørgensen *et al.*, 2001a).

The convergence of several lines of inquiry was crucial for the rapid growth of interest in the issue of endocrine disruption in the 1990s. A number of worrying trends related to human male reproductive health had been reported globally, including decline in semen quality parameters and increases in the incidence of testicular cancer, hypospadias and cryptorchidism. At the same time, adverse trends in the reproductive health of wildlife in some regions outside the Arctic had also been noted and correlated with exposure to environmental contaminants, and in some cases specific chemicals were implicated. Evidence was also emerging from a variety of experimental studies that many widely used chemicals, distributed extensively in the environment, had the ability to bind and activate estrogen receptors. Although their affinity for the receptor was weak compared with either the natural ligand or DES, their activity was regarded as sufficient to support a working hypothesis that environmental chemicals might be damaging the reproductive health of human and wildlife populations by interfering with sex hormone activities. Behind this concern was the suspicion that chemicals acting through hormone receptors might mimic the natural hormones and have profound effects at very low concentrations. The conjunction of threat both to human and wildlife populations led to responses from international organizations (including AMAP), governments, and the chemical industry. The following general needs were identified.

- Further research to confirm the existence of effects from environmental exposure on reproductive health of humans and wildlife.
- In cases where an adverse effect was confirmed, establishment of the causative link to exposure to an environmental chemical.
- Development of reliable methods, and possibly new methods, for detecting chemicals with potential to cause adverse effects (monitoring).
- Ranking of known and suspected endocrine-disrupting compounds for possible regulatory action (prioritization).
- Possible action to limit release of certain chemicals to the environment.

In order to establish consensus on the scope of this issue, to facilitate the identification of active chemicals, and to underpin future regulatory control it is essential to agree on a precise definition of an endocrine-disrupting compound. In 1998, the International Programme on Chemical Safety and the U.S. EPA's Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) proposed the following working definition:

An endocrine disrupter is an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle.

Thus, the term 'endocrine disrupters' covers all kinds of exogenous interfering chemicals; including synthetic chemicals and synthetic and naturally occurring hormones. Exposure can occur via dairy products and food intake, drinking water, and pharmaceuticals, etc. The ability of a chemical to affect humans or wildlife depends on factors such as structure and concentration, bioavailability, degradation/metabolism, and uptake, etc. The observed potency of a chemical is, therefore, very dependent on concentration and the system applied for testing. This can result in several classifications for any one single chemical, for example as carcinogenic, teratogenic, toxic, and endocrine disrupter, depending on which characteristic of the chemical is studied.

Many of the compounds suspected of endocrine-disrupting activity are known to be toxic (in some cases acutely toxic) at higher concentrations. They were therefore banned or controlled in some countries, either on this basis or because of their persistence and capacity to bioaccumulate in biota. Chronic low dose exposure and the subsequent bioaccumulation of lipophilic POPs with long biological half-lives is of special concern. These POPs may over time bioaccumulate to a critical level capable of eliciting an effect. Moreover the 'life-long' duration of exposure, together with increasing environmental levels, may maximize the likelihood of induction of effects. Such factors must be taken into account in sub-chronic, chronic and/or multi-generation tests. Because of the complexity of the endocrine system, and the complex nature of human epidemiological studies, animal studies and *in vitro* screening methods are widely used for toxicity evaluation and risk assessment.

The U.S. EDSTAC has recently developed a strategy for testing chemicals for endocrine modulating activity including an initial sorting of chemicals (based on existing data), priority setting (based on knowledge of exposure), and tier 1 screening and tier 2 testing, comprising:

Tier 1 screening

In vitro assays
- Estrogen receptor binding and reporter gene assays.
- Androgen receptor binding and reporter gene assays.
- Steroidogenesis assay with minced testis.

In vivo assays
- Rodent 3-day uterotrophic assay: increase in uterine weight in ovariectomized rat.
- Rodent 20-day pubertal female with thyroid: age of rats at time of vaginal opening.
- Rodent 5-7 day Hershberger assay: change in weight of prostate and seminal vesicles in castrated rats.
- Frog metamorphosis assay: rate of tail resorption in *Xenopus laevis*.
- Fish gonadal recrudescence assay: effects on light and temperature sensitive sexual maturation.

Tier 2 testing
(Intended to determine and characterize the effects of the chemical on the endocrine system.)
- Two-generation mammalian reproductive toxicity study or a less comprehensive test.
- Avian reproduction test.
- Fish life-cycle test.
- Mysid (shrimp) life-cycle test.
- Amphibian development and reproduction test.

6.1.2. Single compound and chemical mixture exposures

There are a number of factors that complicate the toxicological evaluation of mixtures. First, it is important to remember that no test can evaluate all possible endpoints. However, existing methods in general include numerous endpoints that are sensitive to both strong and weak xenoestrogens such as the reproductive and developmental effects in humans and rodents of DES (Gill *et al.*, 1979; Herbst *et al.*, 1971; McLachlan, 1981; Wilcox *et al.*, 1995) and DDT or chlordecone (Daston *et al.*, 1997). These endpoints, obtained by multi-generation studies in rodents, are sufficient to indicate a hazard. Subsequent decisions to further characterize the cellular and molecular steps in the hazard evaluation require mechanistic research for risk assessment, taking into account the possibility that the observed adverse effects may not be the most sensitive manifestation of toxicity. Second, two or more compounds may have additive effects as a result of acting via the same mechanism in concert. They may also elicit antagonistic (less then additive) or synergistic (greater than additive) effects. Some studies have suggested synergistic responses of steroidal estrogens *in vitro* (yeast) and *in vivo* (turtle) (Arnold *et al.*, 1997a,b). However, estrogenic tests with mixtures of dieldrin and toxaphene in human breast cancer MCF-7 cells, yeast-based human estrogen receptor assays, and mouse uterus tests showed no apparent synergism (Ramamoorthy *et al.*, 1997).

There are several other complications that must be taken into account when generalizing about what is known concerning the toxicity of single compounds and/or mixtures. A compound may have multiple sites of action and its toxicity may be mediated by different mechanisms. Many substances are biotransformed to metabolites (e.g., hydroxylated PCB metabolites) that may have a different biological activity than that of the parent compound. In addition, a single environmental contaminant may induce different effects depending on the organism's age and reproductive state at the time of exposure. Lead is an example of a contaminant having little effect on neurobehavioral function in adults but irreversible effects on intelligence quotient (IQ) and behavior when exposure occurs *in utero* during the development of the nervous system (Carpenter *et al.*, 1998).

It is known that developmental toxicity is dependent on highly susceptible periods of organogenesis, as demonstrated by prenatal exposure to, e.g., DES and thalidomide, and postnatal exposure to Pb, pesticides and radiation (Selevan *et al.*, 2000).

Toxicity scales have been developed for compounds that share a common mechanism of action. This concept was applied to mixtures of dioxin-like compounds that bind the aryl hydrocarbon receptor (AhR). The AhR is an intracellular ligand-dependent transcription factor expressed in most tissues of mammals. Dioxins and furans (polychlorinated dibenzo-*p*-dioxins, PCDDs; polychlorinated dibenzofurans, PCDFs) as well as non- or mono-*ortho* chloro-substituted PCBs are ligands to the AhR (Birnbaum, 1995; Brouwer *et al.*, 1999; Carpenter *et al.*, 1998). The activated ligand-receptor complex triggers the expression of enzymes including P4501A1, P4501A2, P4501B1, glutathione S-transferase, glucuronyl transferase, δ-α aminolevulinate synthethase, epidermal transglutaminase, NAD(P)H:quinone oxidoreductase and aldehyde-3-dehydrogenase, which are involved in metabolism and detoxification of many POPs (Hahn, 1998; Safe and Krishnan, 1995).

A common practice in risk assessment is to calculate the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents (TEQs) for mixtures comprising dioxin-like compounds. TEQs are calculated by multiplying the concentration of each dioxin-like compound by its Toxic Equivalency Factor (TEF), which corresponds to the relative potency of the specific compound in generating an AhR-mediated effect, in relation to that of TCDD, the most potent dioxin-like compound. Consequently, the classical TEQ/TEF risk assessment only accounts for potential dioxin-like properties of a mixture and not other relevant toxicological endpoints such as effects mediated via other receptors and biochemical pathways (e.g., interference with the sex hormones and thyroid hormone systems). For example, *ortho*-substituted PCBs are either weak ligands or do not bind at all to the AhR, therefore either very low or no TEF values are given for these compounds. Recently, however, it was reported that the three most highly bioaccumulated di-*ortho* substituted PCBs (CB138, CB153, and CB180) elicit the potential, *in vitro*, to interfere with cell proliferation as well as the function of the androgen and estrogen receptors (Figures 6·1 and 6·2) (Bonefeld-Jørgensen *et al.*, 2001a). These results emphasize that a full assessment of the toxicological potential of a chemical mixture is much more complex than can be deduced by the use of TEQ values alone.

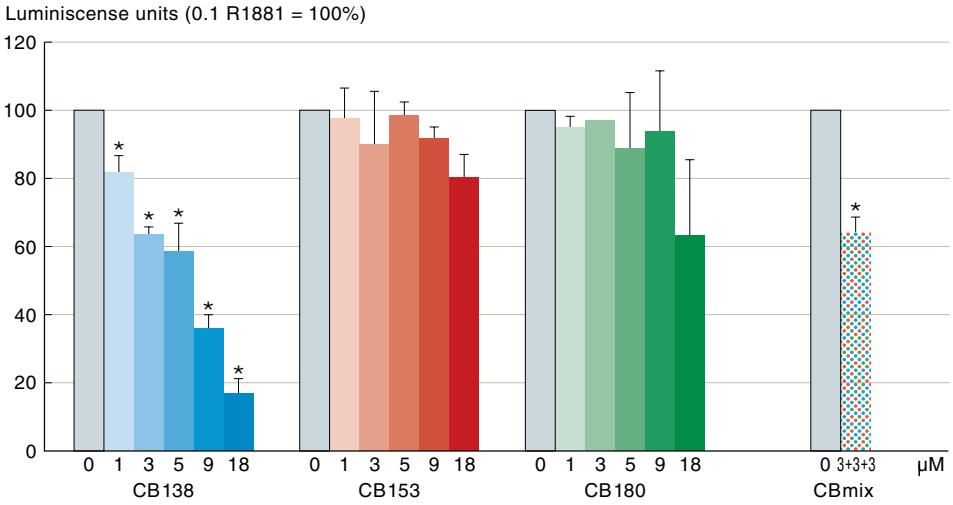

Figure 6·1. Effects of PCB congeners on androgen receptor (AR) trans-activity in Chinese hamster ovary cells (CHO cells). The response of PCBs and the AR agonist methyltrienolone (R1881, positive control) were obtained by transiently co-transfection with the pMMTV-LUC reporter plasmid and the pSVAR0 expression plasmid encoding the human AR. Asterisks indicates statistically significant ($P \leq 0.05$) decrease relative to cells treated with 0.1 nM R1881 (which is set to 100%). Source: Bonefeld-Jørgensen *et al.* (2001a).

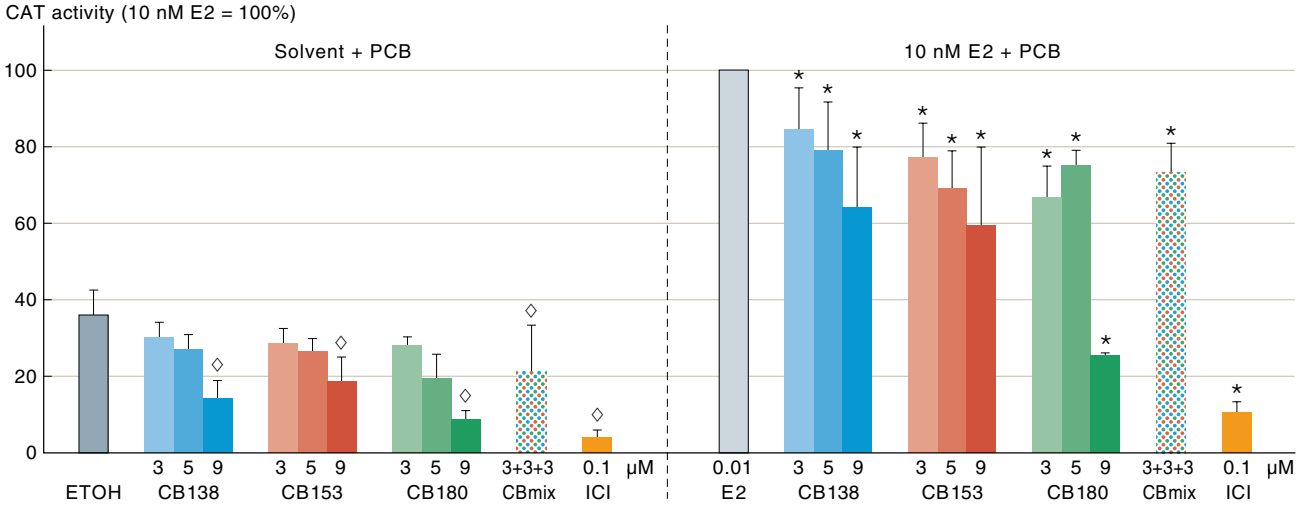

Figure 6·2. Effects of PCB congeners on estrogen receptor trans-activity in human breast cancer MCF-7 cells. The response of the PCBs and the ER agonist 17β-estradiol (E2, positive control) on transactivation of the reporter plasmid pERE-tk-cat in MCF-7 cells. Diamonds indicate a statistically significant ($P \leq 0.05$) difference from the solvent (ETOH) and asteriks from the 10 nM E2 treated cells, respectively. Source: Bonefeld-Jørgensen *et al.* (2001a).

Table 6·1. The AMAP Human Health Effect Monitoring Programme.

Bio-Physical Indicators	Epidemiological Effect Markers	Molecular/Genetic Effect Markers
Health Statistics		
	E Morbidity/Mortality data R Cancer incidence	
Genetic susceptibility studies		
Gene polymorfisms	R Gene polymorfisms	R Genotypes Gene expression (mRNA)
Fertility studies		
Time to pregnancy	R Time or number of menstrual cycles it takes a couple to conceive from discontinuation of contraception	R Receptor/hormone toxicology (estrogenic- and androgenic-like activities are performed in steroid hormone cleared serum samples). R Estrogenic- and androgenic-like activities. Dioxin-like activities in blood serum. *In vitro* hormone receptor bindings.
Semen quality and quantity	R Sperm count/volume Sperm quality/mobility	
Sex hormones (in blood from women and from their male partners providing semen samples)	R Sex hormones	R FSH, Inhibin-B, LH, testosterone, estradiol, sex hormone binding globulin, osteocalcin, pyridolins
Pregnancy outcome		
General indices	E Abortion (spontaneous) Gestational age Birth weight/length Sex (single/multiple) Placenta weight	R Estrogenic-, androgenic- and dioxin-like activities R Cytochrome P450 modulations, DNA adducts
Developmental anomalies	R Maldescent testis Hypospadias Epispadias Ano-genital distance and other indices	
Developmental effects	R Breast milk (POPs, fatty acids)	
Immunological effects		
	R Hospitalization	
	R Vaccination response	R Antibody (HIB)
		R Vitamin A and cytokines, complement system R Dioxin-like activities
Neurological effects		
	R Milestones + age R Pre-school tests: neurophysiological tests neuropsychological tests audiogram and visual tests	R Thyroid hormone R Estrogenic- and dioxin-like activities GSHRd, GSHPx, Ubiquinol 10/Ubiquinone 10, Ox-LDL, F2-isoprostanes

E: Essential; R: Recommended.

The AMAP Human Health Effect Monitoring Programme is designed to examine the effects on reproduction and development of dietary exposure to POPs and heavy metals within the Arctic. One requirement is that a suite of studies are carried out in parallel, including dietary questionnaires and indicators, and contaminant measurements carried out by laboratories with documented QA/QC.

6.2. The AMAP Human Health Effects Monitoring Programme

The broad categories of human health effects that may be linked to exposure to environmental contaminants include: cancer, birth defects, decreased fertility, altered sex hormone balance, immune system defects, neurological effects such as reduced IQ and behavioral abnormalities, altered metabolism, and specific organ dysfunctions (Carpenter *et al.*, 1998). At AMAP Human Health Expert Group meetings held in Ottawa, Canada, September 1999; Rovaniemi, Finland, January 2000; and Tórshavn, Faroe Islands, October 2000, a Human Health Effects Monitoring Programme was recommended to the eight Arctic nations.

The AMAP Human Health Effects Monitoring Programme, as shown in Table 6·1, includes several molecular biomarker endpoints for use in Arctic environmental health studies. Substances commonly found in the environment may have the potential to affect several organ

systems. The diseases listed are identified on the basis of studies of both humans and animals, and in most cases these investigations were focused on a single contaminant. Several of these diseases, when found in a given individual, are difficult to ascribe to a particular exposure (Sharpe, 1993; Sharpe and Skakkebaek, 1993). This is generally the case for cancer, reproductive effects (such as infertility, early birth, etc.), many of the endocrine modulators and nervous system actions. Others are clearly attributable to particular exposures, such as kidney disease following Cd exposure or the loss of particular neurons following MeHg exposure (Carpenter *et al.*, 1998).

The main objective of the AMAP Human Health Effects Monitoring Programme is to characterize the impact of dietary exposure to POPs by monitoring biophysical indicators, and epidemiological and molecular/genetic effect markers (see Table 6·1). These effects studies are directed towards examining the hypothesis of xenobiotic interference with homeostasis of hormone functions, with a special focus on circumpolar populations. A number of persistent OCs exhibit estrogenic (and anti-estrogenic), androgenic (and anti-androgenic) and dioxin-like activities. Some bind to the estrogen receptor (e.g., DDT, toxaphene, CB138, CB153, CB180) (Bolger *et al.*, 1998; Bonefeld-Jørgensen *et al.*, 1997, 2001a), and some bind to the androgen receptor (e.g., DDE, vinclozolin) (Bonefeld-Jørgensen *et al.*, 2001a; Crisp *et al.*, 1998; Kelce *et al.*, 1997; Kelce and Wilson, 1997) or bind to both receptors (e.g., metabolites of methoxychlor, CB138). Dioxins have been characterized as anti-estrogenic due to their AhR-mediated interference with estrogen receptor activities (Kharat and Saatcioglu, 1996; Safe and Krishnan, 1995). Because of the wide variety of endocrine-disrupting effects possibly induced by mixtures of persistent OCs, there is a need to develop markers that integrate the effects of several chemicals on specific hormonal pathways and to combine them with epidemiological studies that are also part of the AMAP Human Health Effects Monitoring Programme.

Sonnenschein and colleagues have devised a method for estimating human exposure to a complex mixture of xenohormones (environmental compounds with hormone-like activities) (Sonnenschein *et al.*, 1995). First, endogenous steroids are separated from persistent OCs in human serum samples by high-performance liquid chromatography (HPLC) and the resulting fractions are tested for estrogenic activity using a proliferation assay with MCF-7 cells. Other investigators have further developed and applied the HPLC fractionation of human serum for separating endogenous hormones from xenohormones to obtain integrative measurements of estrogenic, androgenic, and dioxin-like effects of compounds using reporter-gene cell assays. Recent data, using Inuit serum samples from Greenland, have indicated that the concerted action of accumulated persistent OCs in the human samples exerts an inhibitory effect on the estrogen receptor function in human cells, whereas an increase in AhR activity was observed (Bonefeld-Jørgensen, in prep.).

The morbidity/mortality data and pregnancy outcomes are considered essential effect markers, whereas the other biomarkers listed in Table 6·1 are recommended measurements for inclusion within AMAP monitoring implementation plans. The biophysical indicators and epidemiological and molecular/genetic effect markers included in Table 6·1 are, as far as possible, linked to the different studies. In addition, dietary questionnaires, relevant markers of a seafood based diet (e.g., n-3 fatty acid content in plasma phospholipids), and biomarkers of exposure to POPs should also be included in the studies. Laboratories performing POPs, heavy metal, and lipid analyses must have documented quality assurance / quality control (QA/QC).

Sections 6.3 to 6.8 describe the background and rationale for conducting the health effects studies in the Arctic that are listed in the Human Health Effects Monitoring Programme (Table 6·1). Some studies have already been initiated in some parts of the Arctic, while others are still at the planning phase.

6.3. Genetic considerations

Central to many of the influences on the biological system are effects that occur at the gene level. Genes regulate almost everything, including many aspects of hormonal production and the reproductive system, brain development and function, immune system balances, and organ physiology. A genetic disruption can, therefore, affect different organ systems, as a result of the extensive interactions between these systems; i.e., effects on one organ system may influence the function of other organs. During normal development, genes are activated and deactivated at different stages, often under the control of growth factors and hormones. Environmental factors interfere with these biologically balanced processes and may result in genetic dysfunction. Mutations in genes, inherited or induced by environmental factors, may thus result in reproductive effects, birth defects, and cancer.

Gene polymorphism is known to exist between different ethnic groups, which can result in differences in tolerance, e.g., to food components such as lactose (Harvey *et al.*, 1998; Nei and Saitou, 1986). In addition, gene polymorphism in metabolizing enzymes is suspected to influence susceptibility to environmental carcinogens, affecting the risk of cancer (Autrup, 2000; Coughlin and Piper, 1999; Morabia *et al.*, 2000). Genetic polymorphism and breast cancer risk has been extensively analyzed, and significant differences in genotype frequencies between cases and controls have been found, including the aromatase cytochrome P450 (CYP19) gene which catalyses the conversion of androgens to estrogens (Dunning *et al.*, 1999). Recently, a study suggested an association between PCB concentrations and CYP1A1 gene polymorphism in women breast cancer patients compared to control groups (Moysich *et al.*, 1999).

Serum concentrations of *p,p'*-DDT, *p,p'*-DDE and CB138, CB153, and CB180 were found to be significantly associated to K-ras mutations in exocrine pancreatic cancer (Porta *et al.*, 1999). These results suggest new roles for OCs in the development of several cancers in human beings.

Mitochondrial DNA (mtDNA), which is inherited maternally via the oocyte, has been mapped completely (Thrasher, 2000). The variation of mtDNA can be used

to determine the ancestry of a population. For example, using restriction fragment length polymorphism and DNA sequence technology, the present Inuit from Greenland were shown to be descended essentially from Alaskan Neo-Eskimos; European mtDNA types were not found in the Inuit samples in this study (Saillard *et al.*, 2000). Because of inadequate DNA repair mechanisms, relatively high rates of mutation are accumulated in the mtDNA. Mitochondrial DNA mutations are responsible for several mitochondrial syndromes. A recent study presented a theory of possible linkages between mitochondrial defects and a possible global developmental delay in some children with maternal exposure to environmental OCs. The author suggests that investigators conduct further research into the cause of maternal mtDNA mutations following exposure to mutagenic xenobiotic compounds (Thrasher, 2000).

6.4. Breast cancer

The incidence of breast cancer has increased steadily over the past few decades in women from a number of countries including Finland, Denmark, the United States, and the UK (Hakulinen *et al.*, 1986; Quinn and Allen, 1995). The upward trend is estimated at about 1% per year since 1940. In the Arctic, the prevalence of breast cancer in Greenland Inuit is much lower than in the general population of the Western Hemisphere; however, during the 1990s an increasing incidence was also observed in this population (Nielsen, 2000). The established risk factors, such as genetic inheritance and factors resulting in an increased total lifetime exposure to biologically active estrogens, can explain only about a third of the cases (Davis and Bradlow, 1995). Estrogens have a prominent role in the pathogenesis of breast cancer (Lippman and Dickson, 1989), and it has been hypothesized that xenoestrogens may contribute to the total estrogenic burden.

Several studies on DDE, PCB and hexachlorobenzene (HCB) concentrations in breast cancer patients versus controls have been carried out in Europe, Asia, and North and South America. Overall these studies do not support a major role for exposure to persistent OCs as a risk factor (Laden and Hunter, 1998; Safe, 1997). However, the results are inconclusive, particularly for high-level exposure. Six studies reported elevated levels of DDT or DDE among women with breast cancer. Seven studies found no differences in DDT or DDE level between cases and controls, and one study reported higher DDE levels in serum samples among women with breast cancer (Romieu *et al.*, 2000). The limitation of these studies has been discussed with regard to information on duration of lactation, other potential sources of estrogens, and replacement estrogen therapy. The lack of controlling for other sources of estrogens or potentially confounding factors may have masked a real association between OC exposure and breast cancer (Romieu *et al.*, 2000). In Denmark, Høyer *et al.* (1998) reported a significant dose-related association between accumulation of the pesticide dieldrin and the risk of breast cancer. As previously mentioned, a case-control study in western New York State found an increased risk of breast cancer to be associated with the polymorphism of CYP1A1 among women with PCB levels higher than the control group (Moysich *et al.*, 1999). Thus, the importance of environmental xenohormones in the etiology of breast cancer remains controversial.

Accumulation in the fatty breast tissue and the potential of many persistent OCs to exert estrogenic/androgenic- or anti-estrogenic/anti-androgenic-like effects are hypothesized to promote the cancer process through the modulation of the estrogen receptor regulated responses (Wolff and Toniolo, 1995). Therefore, to reject or verify the hypothesis future studies must include – in addition to the epidemiological investigation and burden of POPs – information on genetic polymorphisms and biomarkers related to the total impact of components with estrogenic (or anti-estrogenic), androgenic (or anti-androgenic), and dioxin-like activities. Currently, a pilot study including these endpoints is being carried out in Greenland (Bonefeld-Jørgensen, pers. comm., 2002).

PCBs and dioxin are well known for their ability to induce certain iso-enzymes of P450 in mammalian liver via the AhR. Some of these enzymes; P4501A1, P4501A2, and P4501B1, are involved in estradiol metabolism and might disrupt hormone levels (Spink *et al.*, 1992a,b, 1994, 1998). *In vitro*, several persistent OCs have been shown to increase the 16α-OHE1:2-OHE1 estradiol metabolite ratio; 16α-OHE1 is regarded as highly estrogenic while 2-OHE1 is a weak anti-estrogen (Bradlow *et al.*, 1995). Some studies have reported higher levels of the 16α-OHE1 metabolite in urine of breast cancer patients (Bradlow *et al.*, 1995; Safe, 2000), whereas other studies did not observe this association (McDougal and Safe, 1998; Ursin *et al.*, 1997). Thus, inconclusive results exist and await further research.

In animal studies the anti-estrogenic capacity of TCDD was suggested to be responsible for a decrease in the incidence of mammary tumors observed in female rats (Kociba *et al.*, 1978). *In vitro* bioassays have suggested that hydroxy metabolites of several PCB congeners also possess anti-estrogenic properties (Moore *et al.*, 1997).

6.5. The reproductive system and fertility studies

The development and maintenance of reproductive tissues is to a large extent controlled by steroidal hormones. Studies *in vitro* or in whole animal model systems have demonstrated that some environmental chemicals either mimic and/or antagonize natural hormone activities. Studies dating back to the late 1960s identified 1-[2-chlorophenyl]-1-[4-chlorophenyl]-2,2,2-trichloroethane (*o,p'*-DDT), a minor constituent of technical DDT, as a weak estrogenic compound capable of causing an augmentation of rat uterine weight in the classic immature female rat model (Bitman and Cecil, 1970). This compound and a few others that share estrogenic properties have been implicated in abnormal sexual development in reptiles (Gaido *et al.*, 1992; Guillette *et al.*, 1994, 1995), birds (Fahrig, 1993; Fry, 1995; Fry and Toone, 1981) as well as feminized responses in male fish (Jobling *et al.*, 1995).

Male reproductive disorders may be mediated by the estrogen receptor; however, they are also consistent with inhibition of androgen receptor-mediated events. Kelce

et al. (1995) identified the major and persistent DDT metabolite, 1,1-*bis*[4-chlorophenyl]-2,2-dichloroethylene (*p,p'*-DDE), as a potent anti-androgenic agent in male rats. In addition to inhibiting androgen binding to the androgen receptor, this compound, when administered to pregnant dams, also induced characteristic anti-androgenic effects in male pups (reduced anogenital distance; presence of thoracic nipples). Treatment with *p,p'*-DDE at weaning delayed the onset of puberty, while treatment of adult rats resulted in reduced seminal vesicle and ventral prostate weights.

TCDD is yet another OC which has been shown to alter sexual development in male rats (Mably *et al.*, 1992). Decreases in epididymis and cauda epididymis weights, decreases in daily sperm production and cauda epididymal sperm number were observed at day 120 and at most earlier times, when a dose as low as 64 ng/kg was administered to dams on day 15 of gestation.

During the differentiation of reproductive organs, hormones, growth factors, and other endogenous mediators regulate gene expression and direct differentiation. The marked difference between exposure to chemicals including endocrine-disrupting compounds during critical periods in development versus during adulthood is the irreversibility of an effect during development. Evidence indicates that changes in concentrations of androgen and estrogen result in permanent changes in cell function. For example, the higher level of testosterone in male mouse fetuses relative to female fetuses results in the differentiation to prostate tissue as opposed to vaginal tissue. In addition, a small increase in total circulating estradiol (50 pg/mL) permanently altered prostate size in mice (Bigsby *et al.*, 1999). Thus it is plausible that disruption of the action of estrogen or androgen during critical periods can lead to permanent alterations in the development of reproductive organs and other tissues with receptors for these hormones.

Animal experiments indicate reproductive toxicity following low-level exposure to persistent OCs. In laboratory animals it has been shown that prenatal exposure to PCBs, PCDD or the DDT-metabolite *p,p'*-DDE is associated with reduced male fertility (Kelce *et al.*, 1995; Peterson *et al.*, 1993; Sager *et al.*, 1987).

In wildlife studies on Baltic grey (*Halichoerus grypus*) and ringed seals (*Phoca hispida*) and on Wadden Sea harbor seals (*Phoca vitulina*) there is strong evidence that PCBs in the food chain had impaired reproductive function resulting in population declines (SCTEE, 1999). Most recently, high levels of POPs have been found in Arctic polar bears (*Ursus maritimus*) in Svalbard (Skaare *et al.*, 2000), and a possible association between the reported incidence of pseudohermaphroditism in polar bears and environmental chemicals has been discussed (Wiig *et al.*, 1998).

More information about effects of POPs on Arctic animals can be found in the AMAP 2002 assessment on POPs in the Arctic (AMAP, 2003c).

6.5.1. Time to pregnancy

Time to pregnancy is a measure of the joint reproductive performance of the parents. Temporal changes in human fertility in relation to body levels of OCs have not been extensively investigated. A time-to-pregnancy study did not reveal delayed conception among consumers of fish from Lake Ontario (Buck *et al.*, 1997), but the duration of exposure was rather short and not quantified by measurements. Results of a Scandinavian time-to-pregnancy study indicate delayed conception related to persistent OC body burdens in smokers but not in non-smokers (Axmon *et al.*, 2000).

6.5.2. Semen quality and quantity

A study of sperm counts conducted worldwide suggested that an annual fall of 0.8% had occurred between 1938 and 1990 (Carlsen *et al.*, 1992). Since then, falling sperm count and quality have been reported in a number of countries (Auger *et al.*, 1995; de Mouzon *et al.*, 1996; Irvine *et al.*, 1996; Van Waeleghem *et al.*, 1996) and a study of testicular morphology in Finland (Pajarinen *et al.*, 1997) suggested a reduction in spermatogenesis between 1981 and 1991. In contrast, no evidence for a decline in sperm counts or quality has been found in a number of locations studied within the United States (Fisch *et al.*, 1996), although considerable geographical variation in sperm counts was observed.

Sperm production is controlled by the sex hormones (Sharpe, 1993; Sharpe and Skakkebaek, 1993), and may therefore be influenced by sex-hormone-mimicking compounds. Certain chemicals lower sperm count in animals and in exposed workers. Dioxins and the pesticide endosulfan are known to lower testosterone levels and produce testicular atrophy in male rats, and dioxin, kepone, 2,4-dichlorophenoxyacetic acid (2,4-D), and dibromochloropropane have been suggested to reduce sperm count in men (Paigen, 1999). A number of other possible factors may affect sperm production, including changes in lifestyle. Intake of selenium (Se) is reported to be falling in the UK and Europe (Rayman, 1997) and dietary deficiency of Se has been suggested as a causative agent of lowered sperm production since selenoenzymes play a role in the maintenance of normal sperm motility, testicular morphology and testosterone metabolism. There is general consensus that, in some countries at least, semen quality and counts have declined. However, taking into consideration the influence of bias in recruitment of study subjects and uncertainty in methodologies (Bromwich *et al.*, 1994; Lerchl and Nieschlag, 1996) it is not known to what extent semen quality reflects incidence of infertility or sub-fertility of males as such.

The only known study on semen quality in the Arctic took place in Iceland. A study of 73 men including 27 men with normal semen, who came to the fertility center because of their wives' fertility problems (52% were of proven fertility), 20 men with idiopathic sterility, and 26 men with poor semen quality, showed no correlation between fertility and persistent OC levels. There was a direct relationship between OC levels in plasma and in semen; with concentrations approximately 20 to 50 times lower in semen. Prevalence of obesity (body mass index (BMI) > 30 kg/m^2) was more than three times higher in the group of men with semen problems, and sperm density correlated to BMI (P = 0.003), raising the possibility that increased prevalence of obesity may partly be involved in the decline in male fertility (Magnúsdottir *et al.*, 2002).

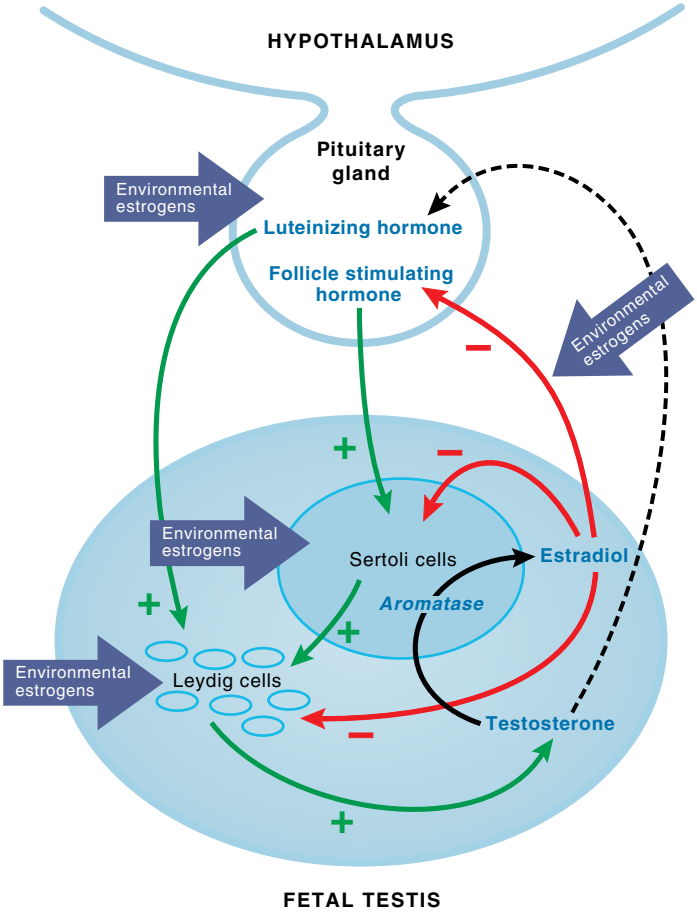

Figure 6·3. Means by which environmental estrogens are thought to disrupt the major hormonal mechanisms involved in growth and function of fetal/neonatal testis. Green (+) and red (–)arrows represent positive and negative feedback mechanisms, respectively. For explanation see the text to the right.

6.5.3. Male reproduction, testicular and prostate cancer

The incidence of testicular cancer has increased quite dramatically in many countries having cancer registries, including Scandinavian countries, the countries around the Baltic Sea, Germany, the UK, the United States, and New Zealand (Adami *et al.*, 1994; Brown *et al.*, 1986; Hakulinen *et al.*, 1986; HMSO, 1992; Wilkinson *et al.*, 1992). Interestingly, the increasing incidence of testicular cancer in Denmark is several times higher than in Finland and further study of this geographical gradient may give important clues on etiology. Prostate cancer incidence has also increased in many countries (Boyle *et al.*, 1995; Merrill and Brawley, 1997). The incidence of congenital malformations such as cryptorchidism (maldescent testis) and hypospadias (malformation of the penis) are thought to be increasing in the Western countries (Berkowitz *et al.*, 1993; Kallen *et al.*, 1986). Testicular cancer has been related to cryptorchidism (Giwercman *et al.*, 1993, 1987) and the testicular maldescent has also been linked to hypospadias (Kallen *et al.*, 1986; Moller *et al.*, 1996; Prener *et al.*, 1996). Moreover it is widely accepted that men with testicular cancer have a higher incidence of impaired spermatogenesis in both cancerous as well as contra lateral testis (Petersen *et al.*, 1998). The increases in the incidence of testicular cancer, hypospadias, cryptorchidism, and reduced sperm count are believed to be associated with *in utero* exposure, i.e., fetal development, and may therefore have common etiology (Sharpe, 1993; Sharpe and Skakkebaek, 1993).

A variety of epidemiological data suggest that the hormonal environment of the fetus may be involved in the development of testicular cancer and congenital malformations. The main evidence, which suggests that prenatal exposure to estrogens has an influence on the development of the male reproductive tract, originates from the treatment of pregnant women with DES. Alteration in estrogen exposure may be influenced by endogenous sources such as a change in diet (low-fiber, high fat, and increase in dairy products) and increase in body fat. Intake and increase of synthetic estrogens such as DES and ethinyl estradiol may occur through the use of oral contraceptive pills, components of which are recycled into drinking water, and also by intake of non-persistent phytoestrogens from plants. Finally, persistent OCs such as PCBs and dioxins are suspected to play a role because of their potential to interfere with hormone homeostasis. In animal studies, TCDD caused changes in both male and female gonadal development and male reproduction (Peterson *et al.*, 1993).

Figure 6·3 summarizes the major hormones which are involved in growth and function of the fetal testis and illustrates how exogenous environmental estrogens may disrupt the normal hormone homeostasis. The basis for susceptibility to adverse effects of estrogen on development of the male reproductive tract centers on the normal hormonal control of the fetal testis.

Sertoli cells play an essential role in spermatogenesis. The production of estrogen and Müllerian inhibiting substance by the Sertoli cells are thought to co-ordinate the processes relating to testicular development and masculinization. Sertoli cells are also thought to regulate the differentiation and multiplication of the early germ cells and fetal Leydig cells and their production of testosterone, which is converted to the more potent androgen 5α-dihydrotestosterone. The follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland control Sertoli cell multiplication and maintain testosterone production by fetal Leydig cells, respectively. A negative feedback system operates in which steroid hormones produced by the testis, testosterone and estrogen, act to regulate FSH and LH and is the basis for the so-called estrogen hypothesis (George and Wilson, 1994; Sharpe, 1993, 1994; Sharpe and Skakkebaek, 1993). Estrogen seems to be necessary in male development and function. In addition, immature Sertoli cells, Leydig cells, and germ cells possess the enzyme aromatase, which is responsible for the synthesis of estradiol from testosterone. Estrogens, including environmental estrogens, have the potential to act via various points within this feedback loop to alter hormone level and testicular function.

Sertoli cells produce inhibin-B in a feedback loop, which regulates FSH secretion. Neonatal secretion of inhibin-B can be used as a measure of Sertoli cell number and a sensitive marker for Sertoli cell toxicants. In *in vitro* responses of immature rat Sertoli cells, estrogens, bisphenol-A and the pesticide lindane were shown to increase the production of inhibin-B, whereas mercury(II) and platinum(II) markedly decreased inhibin-B levels (Monsees *et al.*, 2000).

The extreme sensitivity of the fetus to its hormonal environment is illustrated by studies in mice demonstrating that intrauterine fetal position can influence male sexual behavior and androgen responsiveness (Nonneman *et al.*, 1992). It has been known for some years that DDT has both estrogenic (*o,p'*-DDT) and anti-androgenic (*p,p'*-DDE) isomers (Kelce *et al.*, 1995). However, the range of synthetic chemicals with potential estrogenic activities includes other OC pesticides (Andersen *et al.*; 2002, Kelce *et al.*, 1995; Soto *et al.*, 1994), PCBs (Connor *et al.*, 1997), alkylphenolic compounds (Routledge *et al.*, 1998), phthalate esters (Harris *et al.*, 1997) and bisphenol-A (Ben-Jonathan and Steinmetz, 1998; Gould *et al.*, 1998). All chemicals identified as having estrogenic activity are approximately 10^3- to 10^6-fold less potent than estradiol and recent data suggest that they have similar binding affinity for the ER-α and ER-β estrogen receptors (ER) (Kuiper *et al.*, 1998). Moreover, new data suggest that a number of phthalates and alkylphenol chemicals, which act as weak ER agonists *in vitro*, also elicit weak androgen receptor antagonism *in vitro* (Sohoni and Sumpter, 1998). This complicates the prediction of *in vivo* activity of the chemicals. There is some *in vivo* evidence that high exposure of rats to dibutyl-phthalate during gestation and lactation causes abnormalities of the male reproductive tract (Mylchreest *et al.*, 1999). Processes regulated by testosterone were also affected by dibutyl-phthalate exposure resulting in reduction of anogenital distance, hypospadias testicular maldescent, atrophy and underdevelopment of seminal vesicles and prostate. Almost no effect was observed on the female offspring. These observations are similar to effects of prenatal exposure to the anti-androgen flutamide and the anti-androgenic fungicide vinclozolin (Gray *et al.*, 1994; Kelce *et al.*, 1994; Mylchreest *et al.*, 1999). Also, the herbicide Linuron (3-[3,4-dichlorophenyl]-1-methoxy-1-methylurea) was shown to impair testosterone-mediated reproductive development in rats (McIntyre *et al.*, 2000).

The strongest evidence, which implicates exposure to synthetic chemicals as a factor in reproductive tract abnormalities, comes from wildlife. The best-known endocrine-disrupting compound causing serious reproductive abnormalities is tributyltin (TBT). TBT acts as an anti-estrogen since it inhibits aromatase causing an increase in androgens and masculinization of female marine gastropods (Yamabe *et al.*, 2000). In human prostate cancer cells TBT and triphenyltin (TPT) are shown *in vitro* to stimulate cell proliferation and androgen receptor transcription such as prostate specific antigen (Yamabe *et al.*, 2000). Moreover, a number of polycyclic aromatic hydrocarbons (PAHs) were shown, *in vitro*, to possess anti-androgenic activities (Vinggaard *et al.*, 2000).

In summary, more studies are required to support the involvement of environmental chemicals as a risk factor in the proposed decline in male fertility.

6.5.4. Effect on hormone receptor numbers

The responsiveness of a tissue to a hormone depends on the density of receptors within its component cells. The number of receptors is determined by their rate of synthesis and catabolism, which is in turn controlled by complex feedback mechanisms involving hormone action. Some chemicals are shown to interfere with this regulation. For example, TCDD can act to decrease or increase the expression of the ER (Romkes *et al.*, 1987), and compounds which bind to the ER (e.g., ICI 182,780, toxaphene, CB138 and several pesticides) influence receptor functions as well as the cellular level of ER mRNA (Andersen *et al.*, 2002; Bonefeld-Jørgensen *et al.*, 2001a; Jensen *et al.*, 1999).

6.5.5. Effects on synthesis, storage, release, transport, and clearance of hormones

Hormones, including sex steroids, thyroid hormones and glucocorticoids, are transported bound to carrier proteins and their effects are to some extent influenced by the level of these proteins in the blood. Sex hormone-binding globulin (SHBG) is a plasma glycoprotein that binds certain estrogens and androgens with high affinity. Hormones bound to SHBG are not bioavailable for transport into effector cells. In mammals, estrogen increases the concentration of SHBG in plasma, whereas it is decreased by androgens. Plant estrogens (phytoestrogens) also stimulate SHBG synthesis (Murkies *et al.*, 1998). A positive correlation was recently reported between *p,p'*-DDE concentrations in plasma lipids and plasma concentrations of SHBG in young men exposed to DDT during anti-malarial campaigns in Chiapas, Mexico. *p,p'*-DDE levels were also negatively correlated with bioavailable testosterone concentration, semen volume and total sperm count (Ayotte *et al.*, 2001).

Xenobiotic ligands such as hydroxy-PCBs, phthalate esters and chlorinated pesticides in general either do not bind to SHBG or show low affinity compared to 17β-estradiol (Jury *et al.*, 2000). Although these compounds bind to SHBG with much lower affinity than endogenous sex steroids, these interactions may be physiologically relevant in situations where SHBG levels are high and endogenous hormones are low, such as prepubescent children and women taking contraceptives. Moreover, the lack of binding of xenobiotic ligands to SHBG might cause biologically relevant concentrations in spite of their relatively low concentration compared to endogenous hormones.

6.5.6. Hormone profiles

To obtain a steroid profile of androgens for both precursors and metabolites of dihydrotestosterone (DHT) a series of steroids, including DHT, could be measured: dehydroepiandrosterone (DHEA), androst-5-ene-3β, 17β-diol, androstenedione, testosterone, estrone, estradiol, DHEA sulfate, androstane-3α, 17β-diol glucuronide, and androsterone glucuronide. Information on these steroid levels enables a better understanding of any alteration in steroidogenic enzymes in classical steroidogenic tissues, such as adrenal glands and the testis, and for steroidogenic transforming enzymes localized in peripheral tissues such as the prostate and skin.

In the Arctic, no full studies have yet been conducted on hormone profiles and contaminant exposure. Dewailly and colleagues recently carried out a pilot study in Greenland (n=48 males) and the following male hormones were measured: DHEA, δ5-diol, δ4, testosterone,

DHT, E1 and E2. These hormone levels will be correlated with persistent OC levels after adjustment for age, BMI and smoking (Dewailly, pers. comm., 2001).

6.6. Osteoporosis

Persistent OCs have recently been associated with an increased risk of osteoporosis in humans (Beard *et al.*, 2000). The relationship between DDE and bone mineral density was recently examined in 68 sedentary women who reported adequate dietary intake of calcium. Reduced bone mineral density was significantly correlated with age ($r = -0.36$, $P = 0.004$), as well as with increases in the log of DDE levels in serum ($r = -0.27$, $P = 0.03$). These results suggest that past community exposures to DDT may be associated with reduced bone mineral density in women. As a potent androgen receptor antagonist, DDE may reduce the inhibitory effect on cytokines and result in the inappropriate turnover of osteoclasts or inadequate production of osteoblasts within bone marrow, thus leading to reduced bone density (Beard *et al.*, 2000). Bone metabolism markers that could be used are: serum markers of bone formation (osteocalcin), and calcium resorption (urinary pyridinolines). A study of factors associated with osteoporosis in Greenland women is underway and results will be available in the near future (Dewailly, pers. comm., 2001).

6.7. Pregnancy outcome

6.7.1. Developmental anomalies

Developmental anomalies related to OC burden are discussed in section 6.1.1. There are relatively few reports of possible effects of OCs in humans on abortion, gestational age, and birth weight and length, and in general this issue remains to be elucidated.

6.7.2. Placental biomarkers of *in utero* development

Cytochrome P450 1A1 (CYP1A1) is a phase I biotransformation enzyme expressed in extra-hepatic tissues in humans, and its regulation is mediated by the AhR (Safe and Krishnan, 1995).

Hydroxylation of toxicants by P450s leads to the formation of reactive intermediates that may damage DNA. PCBs were shown to form such reactive intermediates *in vitro* and to form DNA adducts following their bio-activation in hepatic microsomal systems containing high levels of P450s (McLean *et al.*, 1996; Oakley *et al.*, 1996).

CYP1A1 induction and DNA adducts were investigated as possible markers of early biological effects related to OC exposure in Inuit women from Nunavik. CYP1A1-dependent ethoxyresorufin-*O*-deethylase activity (EROD) and DNA adducts were measured in placenta samples obtained from 22 Inuit women from Nunavik. These biomarkers were also measured in 30 women from a Quebec urban center (Sept-Îles) as a reference group. Prenatal OC exposure was determined by measuring these compounds in umbilical cord plasma. Placental EROD activity and the amount of DNA adducts thought to be induced by OC exposure were significantly higher in the Nunavik group than in the reference group. For both biomarkers, smoking was found to be an important confounding factor, but OC exposure was significantly associated with EROD activity and DNA-adduct levels after stratifying for self-declared smoking status. It was concluded that CYP1A1 induction and DNA adducts in placental tissue could constitute useful biomarkers of early effects induced by environmental exposure to OCs (Lagueux *et al.*, 1999).

A second study was conducted to determine whether environmental exposure to PCBs induces placental CYP1A1 in Inuit women. This more recent study was designed to better control the confounding effect of smoking. Cotinine concentration in meconium and Cd concentrations in placenta had previously been validated as markers of prenatal exposure to tobacco smoke (Pereg *et al.*, 2002). Placenta, cord blood, and meconium samples were obtained from 35 Inuit women from Nunavik and 30 women from Sept-Îles (reference population). Efforts were made to sample more smokers in the Sept-Îles population and more non-smokers in the Nunavik population in order to balance the smoker and non-smoker groups. Smoking status was ascertained on the basis of cotinine concentration in the meconium and, when necessary, individuals were re-assigned to the proper smoking category based on this marker. PCB concentrations were measured in cord plasma and EROD was assessed in placenta. Despite the higher PCB exposure of the Inuit population, both groups showed similar EROD activities when the data were stratified according to the smoking status ascertained by the cotinine concentration. In the Nunavik population, EROD activity was correlated with 2,2',4,4',5,5'-hexachlorobiphenyl (CB153) plasma concentration (a marker of exposure to the environmental PCB mixture). However, cotinine concentrations in meconium were also significantly correlated with CB153 plasma concentrations and multivariate analyses failed to demonstrate a significant contribution of PCB exposure to placental CYP1A1 activity when tobacco smoking (as estimated by cotinine concentration in the meconium) was included in the analysis.

In summary, the results from this study do not support the hypothesis that low-level environmental exposure to PCBs induces an increase in CYP1A1 activity in the placenta, and leaves tobacco smoking as the major modulating factor (Pereg *et al.*, 2002).

6.7.3. Birth weight

CYP1A1 was reported to have been induced in the placenta of women who smoked during pregnancy and lower birth weights of newborns were observed for smokers with high placental aryl hydrocarbon hydroxylase (AHH) activity compared to smokers with lower AHH activity (Pelkonen *et al.*, 1979). Placental homogenates from Taiwanese mothers who developed Yu-Cheng disease (and thus had been highly exposed to PCBs and PCDFs; see section 6.8.1.5.) had 100-fold greater CYP1A1-related AHH activity levels than those measured in homogenates from non-exposed mothers (Wong *et al.*, 1985). This enzyme induction was significantly associated with low birth weights (Lucier *et al.*, 1987). A study of Swedish fishermen's wives and 1501 children supported an association between a high consumption of POP-contaminated fish from the Baltic Sea and an increased risk of low birth weight. The women

interviewed from the east- and west coast cohorts ate locally caught fish more than twice as often as their referents. Compared with the regional population, the women in the east coast (Baltic Sea) cohort gave birth to an increased number of infants with low birth weights (< 3000 g), whereas the opposite was seen in the west coast cohort. Infants in the east coast cohort had significantly lower birth weights than infants from the west coast cohort (median 3530 g versus 3610 g, $P < 0.001$) (Rylander *et al.*, 1995). Later studies have strengthened the hypothesized association between exposure to persistent OCs during childhood and adolescence and an increased risk of having an infant with low birth weight (Rylander *et al.*, 1998, 2000). A significant relationship between lower birth weight and PCB accumulation was reported in a study of newborns of mothers who had eaten fish with high PCB contents from Lake Michigan (Fein *et al.*, 1984). However, at background levels of PCBs no correlation between PCB concentration and birth weight has been observed (Rogan *et al.*, 1988).

The association of maternal smoking and blood Hg concentration with birth weight was studied in 1106 live born singletons from Greenland with a gestational period of 37+ weeks (Bjerregaard and Hansen, 1996). Smoking was significantly associated with low birth weight while consumption of marine mammals, and maternal or cord blood Hg concentration were not. In West Greenlanders a weak association was found between Hg and low birth weight. High concentration of Hg and OC substances is suspected to reduce birth weight; however, a positive influence of marine diet on birth weight due to n-3 fatty acids has been reported and may counteract the effect of POPs (Bjerregaard and Hansen, 1996).

6.7.4. Sex ratios

There have been suggestions of alteration in sex ratios following an accidental environmental exposure to dioxin in Seveso (Italy) in 1976. Of 74 births in the most heavily contaminated zone there was an excess of females; 26 males and 48 females were born (Mocarelli *et al.*, 1996). A similar occurrence has been noted for some other high occupational exposures to chemicals, e.g., dibromochloropropane (Safe, 2000). There is evidence from several countries that in the past few decades there has been a small but significant decrease in the male to female sex ratios (Safe, 2000). However, the potential role of male/female sex ratios as an indicator of environmental exposure to hormone-disrupting chemicals is still controversial and awaits further research.

6.8. The neuro-endocrine-immune system

In vertebrate species, the neuro-endocrine-immune system is responsible for many complex, inter-related physiological processes including homeostatic, reproductive and immune functions. There are four main types of hormones: polypeptides, eicosanoids, steroids, and thyroid hormones. The inter-dependency of the neuro-endocrine and immune systems is reflected by the production of hormones, neuropeptides and other neurotransmitters by some immune cells. These hormones play a role in regulation of the immune system, while endocrine and nervous tissues express receptors for many substances produced by the immune system (Marchetti *et al.*, 1995). The major focus of interest in endocrine disruption has been reproduction and sexual differentiation and development, with most attention to effects associated with steroid hormones, and to a much lesser extent, the thyroid hormones.

Gonads primarily produce sex hormones, androgens (male) and estrogens (female), which play vital roles in the control of reproductive functions. The glucocorticoid hormones, such as cortisol, are produced by adrenal glands and have fundamental effects on metabolism, as well as influencing the immune and reproductive systems (Vacchio *et al.*, 1998). Thymulin, a polypeptide hormone, is found mainly in the cortex and medulla of the thymus and is thought to be involved in the education and maturation of the immune system's T-cells. Interference with production of this hormone affects the ability of the thymus to produce mature T-cells. There are thought to be feedback mechanisms linking thymulin to testosterone, estrogen, cortisol and thyroid hormone balance (Marchetti *et al.*, 1995; Marsh and Scanes, 1994).

6.8.1. Immune system functions

The immune system includes a complex network of cells. Tissue cells involve macrophages, mast cells and dendritic cells. The lymphocytes are divided into T- and B-cells, these are subdivided into T-helper cells (Th, CD4) and T-cytotoxic cells (Tc, CD8), plasma cells (CD20) and natural killer cells (NK). The immune system cells communicate via highly regulated interleukine expressions. Macrophages initiate a non-specific immune response by phagoctytoses of allergens and then represent the antigen on their surface for the Th-cells. The Th-cells are then activated and secrete cytokines, which activates other Th-, Tc-, and B-cells. Thus the Th-cells play a central role in the immune system.

The extensive interaction between the immune and nervous systems involves common use of messengers such as neurotransmitters and cytokines (Carpenter *et al.*, 1998).

6.8.1.1. Effects of POPs and metals on the immune system

Dioxins, coplanar PCBs, and PAHs suppress the immune system (Carpenter *et al.*, 1998). Lead, however, affects the immune system differently promoting hypersensitivity, rashes, and auto-immunity. Investigations suggest that the dominance of different populations of Th-lymphocytes is a major factor in an individual's immune responsiveness. Th1-lymphocytes predominate and produce a particular profile of cytokines in individuals with normal immunity, whereas individuals with hyper-immunity (asthma, skin rashes, and auto-immunity) have predominately Th2-lymphocytes producing different cytokines (Carpenter *et al.*, 1998). Environmental exposure to Pb and Hg was shown to alter the balance between the Th1- and Th2-lymphocytes, and contaminant exposure early in life is suspected to cause prolonged abnormalities in immune function. Moreover, children exposed prenatally to DES showed an altered immune function (Carpenter *et al.*, 1998).

Studies have shown that both synthetic and natural estrogens suppress the immune system (Kendall *et al.*, 1992; Osterhaus *et al.*, 1995), and that during pregnancy, the female immune system is naturally suppressed with a decrease in thymulin and an increase in estrogen levels. The presence of estrogen receptors on thymulin producing cells indicates coordination between these two hormones (Kendall *et al.*, 1992). Increased concentration of testosterone has a positive, enhancing effect on the immune system, whereas increase or decrease in thyroid hormones results in negative effects.

6.8.1.2. Effects of POPs on the immune system of laboratory animals

Several OCs elicit immunotoxic effects in laboratory animals and humans, the most potent being substances structurally related to TCDD such as non- and mono-*ortho* chloro-substituted PCBs as well as 2,3,7,8-chloro-substituted PCDD/Fs. In almost all animal species tested, including primates, PCDD/Fs and PCBs produce myelosuppression, immunosuppression, thymic atrophy, and inhibition of immune complement system components (NRC, 1992). Exposure to TCDD during pre- and/or postnatal life results in more severe effects than if the chemical is administered during adult life and in some species it may be a prerequisite for immunosuppression (Hoffman *et al.*, 1986; Vos and Luster, 1989). In fact, available evidence in laboratory animals suggests that the maturation of the immune system is especially vulnerable to the adverse effects of dioxin-like compounds, chlordane, HCB, PAHs and possibly other endocrine-disrupting compounds such as DDT and kepone (Barnett *et al.*, 1987; Holladay and Luster, 1996).

6.8.1.3. Effects of POPs on the immune system of wild mammals

Persistent organic pollutants, such as PCBs and dioxins, can cause a broad range of immunotoxic effects in Arctic wildlife. More comprehensive information on this subject can be found in the AMAP 2002 assessment on POPs in the Arctic (AMAP, 2003c).

Exposure to POPs has been associated with effects on thyroid hormones and even low levels of PCB suppress thyroid hormone in grey seal pups in the (non-Arctic) Baltic Sea (Jenssen *et al.*, 1996). High body levels of PCBs and other OCs have been associated with suppression of the immune system (Reijnders, 1986), and it has been suggested that these compounds may be implicated in mass mortality among sea mammals (e.g., seals, porpoises, dolphins) (Osterhaus *et al.*, 1995) following infection with the phocine distemper virus, morbillivirus. In otters, environmentally exposed to PCBs, a strong negative correlation was observed between vitamin A and PCB concentrations, and a high incidence of infectious diseases was apparent in contaminated animals (Murk *et al.*, 1998).

6.8.1.4. Immunotoxic effects of mercury

Organic and inorganic Hg possess cytotoxic activities for cellular components of the immune system in several species of rodent. MeHg, a form of organic Hg, can alter non-specific defense mechanisms, such as inhibition of NK cell activity in rats and mice. It also decreases the expression of certain activation markers of T-cells (HLA-Dr, IL-2R) (NRC, 1992). Moreover, it has been well demonstrated that MeHg can affect the functions of B-cells and therefore reduce the humoral mediated response (Daum *et al.*, 1993). Exposure to inorganic Hg induces allergies and autoimmune problems in hypersensitive individuals.

6.8.1.5. Effects of POPs on the human fetal and neonatal immune system

Few data exist regarding the potential immunotoxic effects of *in utero* and lactation exposure to PCBs and dioxins/furans. In 1979, a poisoning from ingestion of rice oil contaminated with PCBs and PCDFs occurred in Yu-Cheng, Taiwan. *In utero* exposed children of highly exposed women were shown to have a higher incidence of respiratory symptoms during their first six months of life (Rogan *et al.*, 1988). An increase in the frequency of pulmonary diseases was suspected to result from a generalized immune disorder induced by transplacental or breast milk exposure to dioxin-like compounds, most likely PCDFs (Rogan *et al.*, 1988). In children and young adults accidentally exposed to PCBs and PCDFs ('Yu-Cheng disease'), serum IgA and IgM concentrations as well as percentages of total T-cells, active T-cells and suppressor T-cells were decreased compared to values of age- and sex-matched controls (Chang *et al.*, 1981). In addition to a higher frequency of middle-ear diseases among 8- to 14-year old children born to Yu-Cheng mothers (Chao *et al.*, 1997), the investigation of delayed type hypersensitivity responses further indicated that cell-mediated immune system dysfunction was more frequent among patients than controls.

Studies of 207 Dutch infants (105 breast fed and 102 bottle fed) have suggested that background levels of PCB/dioxin exposure were associated with lower monocyte and granulocyte counts at three months of age and thus influence the fetal and neonatal immune system (Weisglas-Kuperus *et al.*, 1995). Follow-up studies of the children showed that perinatal background exposure to PCBs and dioxins persists into childhood and might be associated with a greater susceptibility to infectious diseases such as recurrent middle-ear infections and chicken pox, and a lower prevalence of allergic reactions (Weisglas-Kuperus *et al.*, 2000).

In Nunavik, an epidemiological study investigated whether OC exposure is associated with the incidence of infectious diseases in Inuit infants and with immune system dysfunction. The number of infectious disease episodes in 98 breast-fed and 73 bottle-fed infants was compiled during their first year of life. Concentrations of OCs were measured in early breast milk samples and used as surrogates for prenatal exposure levels. Biomarkers of immune system function (lymphocyte subsets, plasma immunoglobulins) were determined in venous blood samples collected from infants at 3, 7 and 12 months of age. Otitis media was the most frequent disease with 80% of breast-fed and 81.3% of bottle-fed infants experiencing at least one episode during their first year of life. During the second follow-up period,

the risk of otitis media increased with prenatal exposure to *p,p'*-DDE, HCB and dieldrin. The relative risk (RR) for 4- to 7-month old infants in the highest tertile of *p,p'*-DDE exposure as compared to infants in the lowest was 1.87 (95% confidence interval (CI), 1.07–3.26). The relative risk of otitis media over the entire first year of life also increased with prenatal exposure to *p,p'*-DDE (RR, 1.52; 95% CI, 1.05–2.22) and HCB (RR, 1.49; 95% CI, 1.10–2.03). Furthermore, the relative risk of recurrent otitis media (3 episodes) increased with prenatal exposure to these compounds. No clinically relevant differences were noted between breast-fed and bottle-fed infants with regard to biomarkers of immune function and immunological parameters. It was concluded that prenatal OC exposure can be a risk factor for acute otitis media in Inuit infants (Dewailly *et al.*, 2000b).

In 1997, an international symposium was held in Bilthoven (the Netherlands) to discuss the most appropriate effect biomarkers of immunotoxicity that could be used in epidemiological studies (Van Loveren *et al.*, 1999). Among the conclusions, one of the stronger statements was to use antibody responses to vaccination with an antigen to which no prior exposure occurred. In the scope of the ongoing cohort study on neurodevelopmental effects of Hg and POPs (Muckle *et al.*, 2001a), an immune component was added in 1998. The immune function biomarkers described in sections 6.8.1.6 to 6.8.1.9. were selected based in part on the conclusions of the Bilthoven symposium report.

6.8.1.6. Antibody response following vaccination

Acquired immunity produces a very specific response to a particular microorganism or other type of challenge. It mainly involves the activation of lymphocytes and production of antibodies. Environmental toxins may affect acquired immunity and a broad evaluation of the competence of the response provides a better picture of their effect. The development of disease represents the ultimate endpoint in evaluation of immune suppression. Antibody response to vaccination is an intermediate marker of the competence of the adaptive immunity to infections. Vaccination programmes include essentially three types of products: killed vaccine (influenza, whole cell pertussis, inactivated polio), protein-conjugated or protein-based vaccine (Hemophilus influenza type b (Hib), diphtheria, tetanus, acellular pertussis vaccine, hepatitis B), and attenuated live virus (measles-mumps-rubella, varicella, and the tuberculosis vaccine BCG). Antibody response to conjugated Hib is of great interest. Hib vaccine is important in Inuit children because, prior to immunization, Hib was the most frequent cause of bacterial meningitis in Inuit children, and was five to ten times more frequent than in Caucasian children (Ward *et al.*, 1986).

To evaluate humoral response to Hib-conjugated vaccine, two threshold values of anti-polyribosylribitol phosphate (anti-PRP), a capsule polysaccharide, have been set. An antibody titer of 0.15 µg/mL is indicative of immediate protection against Hib, whereas a titer greater than 1.0 µg/mL was found to protect for longer terms (Ward *et al.*, 1994). It should be noted that Hib vaccination is administered using two doses and that exposure to the natural antigen is used in Nunavik. It is then important to note precisely when the last dose was given prior to the blood puncture (antibodies measurement) (Raby *et al.*, 1996).

6.8.1.7. Complement system

The complement (C') system plays an important role in natural immunity against infectious agents. It is particularly important in young children for whom the acquired immune system is not yet fully developed. Deficiency of many of the C' components is associated with increased susceptibility to infections, generally of the upper respiratory tract. In a murine model, exposure to OCs increased susceptibility to *Streptococcus pneumoniae* infections, decreased C3 levels and lowered total C' hemolytic activity (White *et al.*, 1986).

6.8.1.8. Effects of POPs on cytokine production by Th1/Th2 cells

High levels of OCs and metal ions in blood and tissues are frequently related to fish intake. Fish and seal oil-supplemented diets (rich in n-3 (also called omega-3) fatty acids) have generally been shown to reduce plasma levels of some cytokines (Bonefeld-Jørgensen *et al.*, 2001b). Most human studies have shown decreased plasma levels or diminished production of IL-1 and TNFα, while n-3 fatty acids increased the production of these cytokines in mice (Blok *et al.*, 1996). Both contaminants and n-3 fatty acids alter the balance between Th1- and Th2-type cytokines, and could impair host resistance to infections. IL-1, IL-2, IL-4, IL-6, TNFα and IFNγ have been repeatedly associated with these changes and need to be measured in Arctic populations consuming seafood products. IFNγ and TNFα are known anti-viral cytokines (Zinkernagel, 1993); IL-4 enhances IgG1 and IgE, but reduces IgM production (Ada, 1993); IL-10 down-regulates Th1-cytokines and inhibits IFNγ production (Fiorentino *et al.*, 1989).

One of the principal limitations of using cytokines is their high variability due to minor (usually non-detected) infections. The extremely high incidence of minor infections in the Arctic strongly limits the use of cytokines in epidemiological studies.

Organochlorine compounds and metal ions can modulate the production of Th1/Th2-type cytokines. Two studies using murine leukocytes exposed to metal ions *in vivo* demonstrated that Hg inhibits the *in vitro* production of IFNα, IFNβ and TNFα by macrophages and induces a dose- and time-dependent increase in IL-1 activity (Ellermann-Eriksen *et al.*, 1994; Zdolsek *et al.*, 1994). High blood levels of IL-4 and IgE and low levels of IFNγ have been observed in animal studies involving treatment with Hg (Heo *et al.*, 1996). In humans, occupational exposure to inorganic Hg did not result in a significant variation of the immune response in terms of *in vitro* production of IL-1 and TNFα, whereas a prolonged low-level exposure decreased TNFα concentrations (Langworth *et al.*, 1993). Along with their effects on Th1/Th2-type cytokines, OCs and metal ions are known to alter B-cell activity and to impair host resistance to several

bacterial and viral infections (Heo *et al.*, 1996). In animals, Hg increased by 100-fold the virus titers following infection with herpes simplex virus 2 (HSV-2) and increased by 2-fold the number of macrophages in the heart of mice infected with myocardial coxsackievirus B3 (CB3) 994 (Ellermann-Eriksen *et al.*, 1994; Ilback *et al.*, 1996). Plasma levels of IFNγ in exposed animals were higher than in infected non-Hg-treated mice. In a recent preliminary *in vitro* human whole blood study of induced release of the inflammatory cytokines IL-1β and TNFα upon incubation with various OC substances, 2,3,7,8-TCDD, toxaphene, and CB180 elicited the potential to increase the level of the two cytokines in plasma (Bonefeld-Jørgensen, pers. comm., 2002).

6.8.1.9. **Vitamin A status**

Vitamin A influences the expression of over 300 genes and thus plays a major role in cellular differentiation, including that of cells related to immune response (Semba, 1994; Sommer and West, 1996). Results from different animal and human studies vary; however, almost all studies revealed that lymphopoiesis and/or maturation of lymphocytes are altered (generally reduced) in connection with vitamin A deficiency (Olson, 1994; Semba *et al.*, 1993; Sommer and West, 1996). Vitamin A deficiency could increase frequency, severity, and duration of infections. Diseases in the lower respiratory compartment were associated with vitamin A deficiency in many cross-sectional clinics and population based studies. Also, otitis media was among the first infections to be associated with vitamin A deficiency in humans (Bloem *et al.*, 1990; Semba, 1994; Sommer and West, 1996).

Vitamin A clinical deficiency has never been documented in Canadian Arctic populations. However, a recent report suggests that the daily vitamin A intake in Nunavik falls below the recommended intake (Blanchet *et al.*, 2000). Alteration of vitamin A homeostasis has been associated with PCB exposure in laboratory animals (Ndayibagira and Spear, 1999). Furthermore, POPs such as OCs have been shown to alter the vitamin A homeostasis in many species, including primates (Zile, 1992). It is thus important to better understand the relationships between vitamin A, OC levels, and infectious disease incidence in Arctic populations.

In a pilot study, retinol concentration was measured in umbilical cord plasma of newborns and the vitamin A status was assessed in four populations. The study included 55 First Nations newborns and 56 Caucasian newborns from the middle and the lower north shore of the St. Lawrence River, 135 Inuit newborns from Arctic Quebec, and 22 newborns from the general population of southern Quebec. Mean retinol concentrations in ng/mL were 175.2, 159.5, 148.2, and 242.8, respectively (Dewailly, pers. comm., 2001). These preliminary results may suggest an inverse relationship between retinol concentrations and the POPs burden. The difficulty of using vitamin A as an effect biomarker of PCB exposure is related to (1) the variability of vitamin A intake among individuals, and (2) non-systematic supplementation programmes in infants.

6.8.2. **Neurological system**

6.8.2.1. **Thyroid hormone disruption and neural development**

Thyroid hormones regulate neuronal proliferation, cell migration and differentiation including control of when differentiation begins and when cell proliferation ends (Hamburgh, 1969).

A number of organic and inorganic compounds cause toxic action in the nervous system such as abnormalities of peripheral sensory or motor nerves, resulting in either abnormal or loss of sensation, or muscle weakness (Carpenter *et al.*, 1998). In 1979 it was shown that Pb at very low concentration can cause a decrease in IQ and behavioral problems in children exposed prenatally and in the early postnatal years. Recent studies have suggested that these actions are irreversible. Moreover, several studies suggest that PCBs may have similar effects, with prenatal exposure resulting in decreased cognitive function and behavior that appears irreversible (reviewed by Carpenter *et al.*, 1998).

Although many theories exist as to how PCBs affect neurodevelopment, the main hypothesis involves PCB impact on thyroid hormone homeostasis (Porterfield and Hendry, 1998).

In addition to direct effects on neurons the thyroid system is important to nervous system function. In adults, the thyroid controls the rate of metabolism and neurological functions (Colorado State University, 2000; DeVito *et al.*, 1999; Oppenheimer *et al.*, 1995). In the developing fetus and neonate, the thyroid is essential to organ (e.g., brain) development, the development of the central nervous system, and cell differentiation and growth (Porterfield, 2000; Rodier, 1994). Congenital hypothyroidism results in minimal brain dysfunction, even if treated after birth (Carpenter *et al.*, 1998). PCBs and dioxins and their hydroxylated metabolites have some similar steric features to the thyroid hormones, which enables these compounds to interfere with normal thyroid function. The mechanisms by which PCBs can affect thyroid hormone function, and so influence development, include increase in thyroid stimulating hormone from the pituitary gland, altered structure and increased weight of the thyroid gland, decrease in thyroid hormone, thyroxine (T4) and triiodothyronine (T3), and blockage of binding of thyroid hormone to transport proteins and thyroid receptors (Carpenter *et al.*, 1998).

6.8.2.2. **Effects of POPs on fetal and neonatal neurological capabilities**

There is mounting evidence that environmental background exposure to PCBs, dioxins and furans is sufficient to affect thyroid homeostasis and neurological capability. Studies of Japanese breast-fed neonates (Nagayama *et al.*, 1998) and Dutch children (Koopman-Esseboom *et al.*, 1994b) have shown that these compounds in maternal milk affect the thyroid hormone status in children. Estimated TEQ was significantly and negatively correlated with the levels of T4 and T3 in the blood of breast-fed babies. A follow-up study of the same group of Dutch children found that exposure to high levels of PCBs and dioxins *in utero* and in maternal milk correlated negatively with the cognitive score of the children (Patandin *et al.*,

1997). This is in accordance with the findings of intellectual impairment of children prenatally exposed to PCBs through their mother's intake of polluted fish from Lake Michigan (Jacobson and Jacobson, 1996). Longnecker *et al.* (2000) did not find a significant association between *in utero* PCB exposure among 160 North Carolina children and serum thyroid measured in umbilical cord sera. Nor did a study of 182 children from the Faroe Islands, where marine food includes pilot whales, find any correlation between intellectual function and PCB levels. Maternal serum, hair, milk and umbilical cord blood were analyzed for contaminants. Levels of essential fatty acids, Se, and thyroid hormones were determined in cord blood. The neurological optimality score of each infant was determined at 2 weeks of age adjusted for gestational age, and predictors were assessed by regression analysis. Thyroid function was found to be normal and not associated with PCB exposure (Steuerwald *et al.*, 2000). However, a mild decrease in neuropsychological test scores for children 7 years of age was correlated with prenatal MeHg exposure deduced from maternal hair (10–20 µg/g) compared to controls (3 µg/g) (Grandjean *et al.*, 1997). A later study by Grandjean *et al.* (2001) reported neurobehavioral deficits associated with MeHg in 7-year old children prenatally exposed to seafood neurotoxicants. This study involved analyses of cord blood from 435 children from a Faroese birth cohort. A possible interaction between PCBs and MeHg was noted in this study (Grandjean *et al.*, 2001). In addition, MeHg neurotoxicity in Amazonian children living downstream from gold mining activities has been reported (Grandjean *et al.*, 1999b). Moreover, a cross-sectional and prospective dataset from the Maastricht Aging Study suggests that exposure to pesticides, but not metals and organic solvents, was associated with increased risk of mild cognitive dysfunction in adults (Bosma *et al.*, 2000). A developmental study involving a cohort of 7000 children carried out in the Seychelles found no clear evidence for consistent adverse effects on six developmental outcomes of pre- and postnatal study exposure to MeHg (Axtell *et al.*, 2000).

In a recent study, involving 171 healthy German mother–infant pairs, the effect of prenatal and perinatal exposure to PCBs (estimated as the sum of CB138, CB153, and CB180) on prospectively measured psychodevelopment in newborn infants at age 7, 18, 30, and 42 months was estimated (Walkowiak *et al.*, 2001). In summary, the findings showed that the PCB concentration in serum samples at 42 months increased markedly with duration of breast feeding, up to five times higher than in the group of non-breastfed children. Moreover, prenatal and postnatal exposure to European background PCB levels (1.22 µg/L serum at age 42 months) was associated with a decrease in mental and motor development up until 42 months of age. In comparison, the mean of the sum of these three congeners in the blood of women of child-bearing age from Greenland is 5.0 µg/L plasma (with a range of 1.55 to 9.4 µg/L, depending on the region, see Table 5·2).

6.8.2.3. **Animal studies**

The finding of intellectual impairment in humans is supported by animal studies on exposure to PCBs (Brouwer *et al.*, 1998; Eriksson and Fredriksson, 1998; Hany *et al.*, 1999; Hussain *et al.*, 2000). In animal experiments, several chemicals such as PCBs, flame retardants, pesticides, phthalates, and dioxins have been shown to have the capacity to lower T4 levels in blood (Brucker-Davis, 1998; Fowles *et al.*, 1994).

6.8.2.4. **Thyroid effect studies of Inuit populations exposed to POPs**

In Nunavik, a cord blood monitoring programme took place between 1993 and 1996. Measurements of thyroid hormones were performed on 466 Inuit newborn umbilical cord blood samples. Free T4, total T3, thyroxine-binding globulin (TBG) and thyroid stimulating hormone (TSH) were measured. Hydroxylated metabolites of PCBs (OH-PCBs) and other phenolic compounds were also measured in a sub-sample (n=10). As expected, birth weight was positively associated with thyroid hormones (T4, TBG). For this reason, further analyses were adjusted on birth weight. After adjustment, TBG and TSH were significantly and negatively associated with PCB congener levels (Dewailly, pers. comm., 2001).

The main mechanism for the transport of thyroid hormones to the brain requires them to pass through the blood brain barrier via a thyroid hormone transport protein called transthyretin (TTR) (Chanoine and Braverman, 1992). Although PCBs show some binding affinity for TTR (Chauhan *et al.*, 2000), OH-PCBs have much higher *in vitro* binding affinities that can be as high as 12 times the binding affinity of the natural ligand, thyroxine (T4) (Brouwer, 1991; Cheek *et al.*, 1999; Lans *et al.*, 1994). Binding to TTR is not limited to OH-PCBs. Other chlorinated phenolic compounds such as pentachlorophenol (PeCP), halogenated phenols, and brominated flame retardants (Meerts *et al.*, 2000; van den Berg, 1990; van den Berg *et al.*, 1991) also have strong affinities for TTR. Recently, PeCP was found to be the dominant phenolic compound determined in Inuit whole blood (Sandau *et al.*, 2000a). Thus, other halogenated phenolic compounds may also be important contaminants in plasma as they have been found to exhibit similar toxicological properties to OH-PCBs (Schuur *et al.*, 1998; van den Berg *et al.*, 1991).

PCBs have previously been measured in umbilical cord plasma; however, few studies have examined levels of hydroxylated metabolites in blood, especially in humans. OH-PCBs have recently been quantified in whole blood of Inuit from northern Quebec, Canada (Sandau *et al.*, 2000a), and Swedish and Latvian fish eaters (Sjodin *et al.*, 2000). One study examined chlorinated phenolic compounds in umbilical cord plasma to determine possible differences among three human populations with different PCB exposures due to cultural differences in dietary habits (Sandau *et al.*, 2002). Retinol and thyroid hormone status (triiodothyronine (T3)), free T4, TSH, and TBG were determined in most samples. An inverse association was found ($r = -0.62$; $P = 0.003$) between log-normalized free T4 and log-normalized total phenolic compounds (sum of PeCP and OH-PCBs). Total chlorinated phenolic compounds were also negatively associated with T3 ($r = -0.48$, $P = 0.03$) (Sandau *et al.*, 2002). The results indicate that PCBs, OH-PCBs and PeCP affect thyroid hormone status.

6.8.2.5. Oxidative stress induced to the nervous system by methylmercury

Methylmercury is a highly toxic environmental neurotoxin that can cause irreparable damage to the central nervous system (Choi, 1989; Clarkson, 1993, 1997). Although the underlying biochemical and molecular mechanisms that lead to impaired cell function and nerve cell degeneration are not well understood, there is abundant evidence supporting the hypothesis that a major mechanism of MeHg neurotoxicity involves an oxidative stress (Sarafian and Verity, 1991; Yee and Choi, 1996). Mercury increases production of reactive oxygen species via deregulation of mitochondrial electron transport as well as through glutathione (GSH) depletion (Lund *et al.*, 1993). The oxidative stress hypothesis is clearly supported by the finding that MeHg neurotoxicity can be inhibited by various anti-oxidants including Se (Park *et al.*, 1996) and N-acetyl-L-cysteine, a precursor of GSH (Ornaghi *et al.*, 1993).

Glutathione peroxidase (GSHPx) and glutathione reductase (GSHRd) activities were measured in blood samples from 142 Inuit from Sallummiu, Canada (Mirault and Dewailly, pers. comm., 2001). Activities of enzymes involved in detoxification of free radicals were measured in order to investigate relationships between Hg, Se and oxidative stress. It was observed that Hg was negatively correlated with GSHRd activity; an NADPH-dependent enzyme that regenerates glutathione from glutathione disulfide. In contrast, plasma Se concentration was positively correlated with GSHPx activity; a selenoenzyme that catalyses the conversion of hydrogen peroxides to water. Hence, Hg exposure may diminish defense mechanisms against oxidative stress by limiting the availability of glutathione, while Se may afford protection by favoring the destruction of hydrogen peroxide (Mirault and Dewailly, pers. comm., 2001).

Biochemical assessment of oxidative stress markers also includes three other indices. Firstly, the ratio of the reduced form of coenzyme Q10 (ubiquinol-10) to the oxidized coenzyme Q10 (ubiquinone-10) in plasma, which is now considered as one of the most reliable and sensitive indices of an oxidative stress *in vivo* (Finckh *et al.*, 1995; Lagendijk *et al.*, 1996; Yamashita and Yamamoto, 1997). In contrast to the total level of coenzyme Q10, which is reported to be associated with multiple factors including gender, age, and cholesterol and triglyceride levels (Kaikkonen *et al.*, 1999), the ubiquinol-10/ubiquinone-10 ratio index is apparently independent of these variables and thus represents the oxidative stress index of choice. Secondly, an increased level of specific F2-isoprostanes (direct oxidation metabolites of arachidonic acid) in plasma and/or urine is another index recently used to demonstrate oxidative stress in several pathological conditions involving oxygen free-radical formation (Patrono and FitzGerald, 1997; Pratico, 1999). The most easily measurable and frequently used F2-isoprostane species as a marker of oxidative stress *in vivo* is 8-isoprostaglandin F2-α (Patrono and FitzGerald, 1997; Pratico, 1999). Thus the levels of 8-isoprostaglandin F2-α in plasma samples will be measured. Finally, the level of plasmatic low-density lipoprotein (LDL) oxidation could also be assessed as a potential marker of oxidative stress (Dewailly, pers. comm., 2001).

6.9. Conclusions and unanswered questions

There is increasing evidence of adverse trends in human reproductive health, most notably testicular cancer and female breast cancer, whereas the decrease in sperm counts apparent from some studies is still being discussed. However, causal links between effects and exposure to environmental chemicals have still not been firmly established.

Environmental chemicals have been focused on because of their capacity to interfere with hormone activities and hence their possible relation to trends in hormone related health effects. In wildlife, there is more convincing evidence of links between environmental exposure and endocrine disruption. This strengthens the concerns about endocrine modulation by environmental chemicals in humans. Because the developing fetus is particularly susceptible to exposure to environmental chemicals, and because there are many different effect targets, evaluation in terms of both lifetime effects (generations) and effects on organs (time to dysfunction) is complicated. Much research and monitoring are still required, and there is a need to develop, refine, and validate test methods that can accurately predict the effects of chemicals on human health.

In this context, risk assessment must include interaction between chemicals, because in general humans are exposed to chemical mixtures. Finally, and most importantly, is the question of whether the available evidence is strong enough to warrant regulation of the chemicals concerned: Which test methods should be included for the definition of an endocrine disrupter? Is demonstrating detrimental effects in wildlife sufficient to require regulation of a chemical? International controversy will continue until commonly accepted grounds for regulation of potential hormone-disturbing chemicals are established.

After reviewing what has been done, and what is possible and desirable to do in order to obtain a better assessment of the human health impact related to the contamination of the Arctic food web, implementation of a circumpolar biomarker monitoring and research programme (based on Table 6·1) has been recommended by the AMAP Human Health Expert Group. This set of biomarkers will produce the information necessary to complement and support the determination of causality in relationships found in epidemiological studies. Some of the included biomarkers are also likely to be used in the future to improve risk assessments and to establish guidelines which, at present, are still largely based on experimental studies conducted on laboratory animals. Finally, such a programme will effectively meet AMAP requirements for monitoring human health impacts of contamination in the Arctic.

At the same time, it is important to be aware that the use of biomarkers of effects presents new and difficult challenges in relation to the communication of results to local people. The concept of biomarkers is usually difficult to understand for the lay people, and results can be hard to describe and communicate in an unambiguous manner. The fact that biomarkers are indices of subtle deleterious effects, with variable sensitivity and specificity to disease, makes their interpretation even more difficult. An analogy with the effects of alcohol con-

sumption over time might be useful in helping to illustrate the role that biomarkers of effects can play in describing the development of health impacts (i.e., the sequence from alcohol intake, liver enzyme increases, to development of cirrhosis).

There are still major gaps and deficiencies in our understanding of health effects of food chain contaminants in the Arctic. Some of these are highlighted as follows.

Exposure during the developmental period is important because it represents the most sensitive period for several health effects. However, other critical life stages such as puberty and aging can be highly relevant for effects on reproduction, the immune system, or the nervous system.

Efforts should be made to relate molecular or biological markers to adverse health endpoints at the individual and population level. Biomarkers of effect should be integrated in epidemiological studies in order to fill knowledge gaps between exposure and overt clinical effects.

Although receptor-based assays can be very useful, it is imperative to recognize that endocrine disruptive effects can also be mediated through interactions at other levels (e.g., co-activator or repressor levels, enzymes involved in hormone biosynthesis or degradation, etc.). It will be important to design assays that can be directed toward improved understanding of the mechanism(s) involved, further helping in the interpretation of the results and future prevention.

It is important to recognize that genetic variability may affect the susceptibility of individuals or populations to the effects of POPs and MeHg.

Studies on environmentally relevant mixtures (at relevant concentrations) are required to investigate possible interactions between components of the mixture.

Understanding the relationship between subtle biological effects and chronic diseases may prove to be the greatest challenge. Many potential mechanisms of action have yet to be discovered and researched.