Chapter 3

Toxicology

3.1. Toxicokinetics

3.1.1. Distribution

The majority of the substances dealt with in this report are lipophilic, stable, and persistent. They are taken up by aquatic living organisms via diffusion over the gills and from food in the gastrointestinal tract. POPs, particularly organohalogen substances, cross the gill/gut membrane and enter the blood where they are quickly distributed to high lipid tissues such as the liver and adipose tissue. Metabolism and elimination are often slow, leading to a net increase of these substances in the organism over time (bioaccumulation).

There are species differences in the tissue distribution of POPs, partly due to differences in lipid distribution. Several examples of this were given in the first AMAP assessment (de March *et al.,* 1998). Lipid dynamics can also affect the distribution of POPs. For example, many Arctic animals go through dramatic periods of fat accumulation followed by long periods of fasting. Lipophilic POPs will be sequestered in the fat. As the fat is utilized for energy during fasting, POP concentrations in the remaining fat will increase, driving new equilibria to be established between fat, blood lipids, and the lipids of other organs, effectively redistributing POPs to other compartments in the organism. This implies that different tissues in different species will be targets for possible effects from POPs.

For example, lipid dynamics and resultant redistribution of OCS and several PCBs have been studied in wild anadromous Arctic char. Lipid composition was studied in descending (May) fish, ascending (mid-July) fish and in fish caught in mid-July but held in captivity until late September. From May to July, lipid stores increased five-fold with 50% of the lipid content being in the carcass and 35-50% in muscle (Jørgensen *et al.,* 1997a; Jobling *et al.,* 1998). Most of this was triacylglycerols (TAG). Body lipids decreased from mid-July to September in maturing char by 30-40% from all lipid depots, but the major mobilization was of TAG from the carcass and muscle depots. For mature females, the ovaries contained more than 25% of the remaining lipids but for males, the testes only contained 3% of the remaining lipids. Females lost approximately 80% of their body lipids during spawning and overwintering, whereas males only lost 50-55% of body lipids.

The distribution of OCS was studied in lean and fat char. A higher proportion of the body burden was found in extra-adipose organs such as the liver (2 times) and brain (4 times) of lean char than of fat char (Jørgensen *et al.,* 1997b). Similar results were obtained for PCB distribution in a study where wild anadromous char were captured when ascending and treated with PCBs in September (Jørgensen *et al.* 2002a). The fish were not fed during the winter and PCB and lipid analyses were done at three time periods during the winter and spring. From October to May, there was a 20% decrease in PCB concentrations in the carcass but brain PCB concentrations increased six-fold and liver concentrations doubled over the same time period. These results show a net redistribution of PCBs from the carcass lipids to the brain and liver during periods of stored lipid utilization.

Significant differences were seen in PCB concentrations with higher concentrations in blubber of molting (lean) harp seals (*Phoca groenlandica*) than in pre-weaning (fat) individuals (Kleivane *et al.*, 2003). When the seals were compared on a total blubber burden basis, however, there were no significant differences (i.e., the same amount of PCB was present in the blubber but was more diluted in the greater blubber mass of the obese seals). In another study on harp seals, POP concentrations were measured in blood and blubber of seals before and after a four-week fast, and in wild seals sampled before the breeding season (fat) and during the molt (lean) (Lydersen *et al.*, 2002). In the fasting experiment, POP concentrations in blubber did not change but blood POP levels increased during the fast. In the wild seals, POP concentrations in both blubber and blood were higher in the lean seals than in the fat seals.

Polar bears show seasonal dynamics in OC concentrations related to fasting (Polischuk, 1999; Norstrom, 2000). The concentrations of ΣCBz, ΣPCB, and Σchlordane (ΣCHL) in fat increased and ΣDDT decreased during 47-68 days of fasting and this was entirely due to lipid utilization. For a more thorough presentation of this material, see Section 4.4.7.

3.1.2. Metabolism and elimination

Metabolism of xenobiotics occurs mainly in the liver via a two-phase process. These processes are catalyzed by liver enzymes such as the cytochrome P450 containing monooxygenases (Nebert and Gonzalez, 1987). Lipophilic substances that are resistant to metabolism will be selectively accumulated in living organisms. In addition to detoxification, the enzymatic processes can also create reactive intermediates that may be mutagenic and/or carcinogenic, or metabolites that are stable to further metabolism, that are lipophilic and retained, or that are biologically active, with the ability to bind selectively to proteins and accumulate in the organism.

Many POPs form metabolites that are biologically active. DDT is metabolized in living organisms to DDE, which is lipophilic and toxic, and accumulates in biota (WHO, 1989a). In some cases, a methyl sulfone ($MeSO_2$) group is added during metabolism and a number of $MeSO_2$-DDE and $MeSO_2$-PCB congeners have been identified in animals including several Arctic species (Jensen and Jansson, 1976; Lund *et al.,* 1988; Haraguchi *et al.,* 1990; Bergman *et al.,* 1992b;1994b; Brandt *et al.,* 1992; Haraguchi *et al.*, 1992; Letcher *et al.*, 1995a; 1995b; 1998; 2000b). $MeSO_2$-DDE has a high binding affinity for the adrenal cortex and is highly toxic to this

tissue in mice (Lund *et al.*, 1988; Jönsson *et al.*, 1991; 1992; Brandt *et al.*, 1992; Lindhe *et al.*, 2001). This DDT metabolite is suspected of being one possible cause of adrenal hyperplasia seen in Baltic Sea grey seals (*Halichoerus grypus*) (Jensen and Jansson, 1976; Bergman and Olsson, 1985). $MeSO_2$-PCBs may also play a role in hyperadrenocorticism in Baltic Sea seals (Bergman *et al.*, 1992a; Mortensen *et al.*, 1992).

Previously, research has shown that several PCB congeners also form hydroxylated metabolites (Jansson *et al.,* 1975). This type of metabolite has been found to bind selectively to transthyretin (TTR), one of the major transport proteins for thyroid hormones in the blood (Brouwer *et al.,* 1988; 1990; 1998; Bergman *et al.,* 1994a; Letcher *et al.,* 2000a). TTR is complexed to retinol-binding protein (RBP), which transports vitamin A (retinol). Other POPs have also been found to form hydroxylated metabolites, and are suspected of being biologically active in a manner similar to the hydroxylated PCBs. For example, studies have found that some PBDE congeners form hydroxylated metabolites (Klasson Wehler *et al.,* 1996; Örn, 1997; Örn and Klasson Wehler, 1998; Meerts *et al.*, 2000; Klasson Wehler *et al.*, 2001; Mörck *et al.*, 2003; Hakk *et al.*, 2002). 4-Hydroxyheptachlorostyrene has been identified as a metabolite of OCS in polar bear and ringed seal plasma and has been found to bind to human TTR *in vitro* (Sandau *et al.,* 2000). Pentachlorophenol, a metabolite of HCB, also binds to TTR (van den Berg, 1990). Toxaphene congeners Parlar 32 and 62 are metabolized to hydroxylated metabolites by seal liver microsomes (van Hezik *et al.,* 2001).

TBT is metabolized by the cytochrome P450 system in mammals and fish to DBT and MBT, both of which are biologically active (Fish *et al.,* 1976; Kimmel *et al.,* 1977; Lee, 1991; Martin *et al.,* 1989; Fent and Stegeman, 1993).

PAHs are metabolized by the cytochrome P450 system to reactive intermediates that covalently bind to macromolecules such as DNA and proteins, creating adducts.

Chlorinated paraffins (CPs) have been shown to be biotransformed in fish (Fisk *et al.*, 2000), birds (Darnerud and Brandt, 1982), and mammals (Darnerud, 1984), with the susceptibility decreasing with increasing carbon chain length and chlorine content (Tomy *et al.*, 1998). It would appear that there are a number of metabolic pathways that can degrade CPs, but chlorine content and carbon chain length can influence which pathway is utilized. There is little information, however, on the enzymes involved in the degradation. Although older reports suggested that CPs induce phase I (mixed-function-oxygenase enyzmes, e.g., CYP450) and phase II enzymes (conjugation reactions, e.g., mercapturic acid synthesis) (Haux *et al.,* 1982), more recent work has failed to find CYP1A induction in fish despite high CP exposures (Fisk *et al.*, 1996).

The major excretion route of POPs and their metabolites is via feces and to some extent, urine. Some of this is passive diffusion over the gut membrane and some from bile excretion of metabolites. In invertebrates and fish, excretion of low log K_{ow} compounds may also occur by diffusion through the gill membranes. Female fish and birds excrete lipophilic, organohalogen POPs via their eggs, and female mammals via placental transfer to the fetus and in breast milk. A particular characteristic of Arctic marine mammals is that most have very high fat contents in breast milk in order to facilitate rapid growth in the young during the short growing season. For example, polar bear milk has a fat content of 20-46% (Derocher *et al.,* 1993; Oehme *et al.,* 1995a; Polischuk *et al.,* 1995; Bernhoft *et al.,* 1997) and various seal species have milk fat contents of 30-60% (Addison and Brodie, 1977; 1987; Bacon *et al.,* 1992; Pomeroy *et al.*, 1996; Beckmen *et al.,* 1999). Therefore, excretion of POPs via milk is more important than placental transfer for adult females of marine mammal species. This in turn enhances POP exposure of young, particularly for polar bears, Arctic foxes (*Alopex lagopus*), whales, and seals.

For example, in Alaska, northern fur seals (*Callorhinus ursinus*), pups have significantly higher blood concentrations of several POPs when compared to their dam's blood and milk (Beckman *et al.,* 1999). The pups of younger dams (primaparous) have much higher concentrations than those of older dams (multiparous). Young harp and hooded seals (*Cystophora cristata*) have as high levels of some POPs as their mothers at the end of the lactation period (Espeland *et al.,* 1997). In Steller sea lions (*Eumetopias jubatus*), 80% of the POP burden may be transferred from a female to her first offspring via lactation (Lee *et al.,* 1996). Young polar bears (1-2 years) have PCB levels similar to adult females with high PCB levels (Bernhoft *et al.,* 1997) and polar bear cubs-of-the-year have higher concentrations of many POPs than their mothers (Polischuk *et al.,* 1995). This is of concern, as young animals may be more sensitive to the effects of POPs than adults.

Polischuk *et al.* (2002) also found clear evidence of sex-specific metabolism in the polar bear. Adult male polar bears continued to lose body burdens of chlordane compounds during a three-month fast, indicating metabolism, while non-lactating females did not lose any of their body burden. On the other hand, lactating females lost PCBs faster than males. This results in higher PCB levels, but lower chlordane levels, in male than female polar bears.

A special case is excretion of TBT, which distributes not only to internal organs but also to bird feathers and seal fur (Tanabe, 1999). The yearly molts of birds and seals may be an important excretion pathway for butyltins. Guruge *et al.* (1996) estimated that up to 25% of the body burden could be excreted in cormorants during a complete molting cycle. Comparable butyltin concentrations are seen in male and female marine mammals, indicating that these are not transferred from mother to fetus/pup to the same degree as other POPs (Tanabe, 1999).

The net result of uptake, distribution, metabolism, and excretion will determine the POP levels found in an organism. This is in turn affected by other factors. Studies carried out to determine the uptake, distribution, metabolism, and excretion of POPs usually investigate one substance at a time. Wildlife and humans, however, are exposed to complex mixtures of POPs.

Very little is known about how different POPs affect each other's toxicokinetics. POPs that induce the hepatic cytochrome P450 system will affect the metabolism of

other xenobiotics, for example. This may lead to an increase in xenobiotic metabolism, thus increasing excretion. An increase in xenobiotic metabolism may also lead to an increase in the formation of reactive intermediates, with increased toxicity and tissue damage (Boon *et al.,* 1992). An example of this is the bioactivation of benzo[a]pyrene (B[a]P) and 7,12-dimethylbenz[a]anthracene (DMBA) in animals after exposure to the CYP1A inducer, CB126. Bioactivated B[a]P and DMBA metabolites were found to bind irreversibly to endothelial cells in certain arteries, veins and capillaries of mice, rats and chicken embryos (Granberg, 2001; Granberg *et al*., 2000; Annas *et al*., 1999; 2000).

TBT exposure has been shown to lead to the degradation of cytochrome P450 proteins (CYP1A, CYP2B and CYP3A) and concomitant inhibition of enzyme activities in the liver. This in turn would inhibit the metabolism of organohalogen compounds if an organism was exposed to both substance groups simultaneously, leading to higher accumulation of organohalogens, and possible risk of toxic effects.

Thus, it is very difficult to evaluate the toxicokinetics of environmental exposures to mixtures of POPs. The interactions that have been seen indicate that the relative amounts and the composition of various contaminants in animals may partly be the result of selective effects on the organism's uptake, metabolism, and excretion, and not solely a result of the specific pollution burden of any single contaminant in the area.

3.2. Types of effects

In most laboratory experiments studying the toxicological effects of POPs, animals are exposed to single substances or to technical products, often at acutely toxic doses. In a few studies, combinations of a few substances have been used. It should be remembered that wildlife are exposed to complex mixtures of POPs, most often at low doses, and these mixtures may not resemble the original technical products released into the environment because of weathering processes. There are often considerable species differences in sensitivity to specific POPs as well as differences in response. It is, therefore, often difficult to generalize results found in one species to other species. This is particularly difficult when extrapolating effects seen in controlled studies in laboratory species such as rodents to effects seen in marine mammals in the wild. Other factors such as fat dynamics, delayed implantation, differences in physiology, and toxicokinetics may make wildlife more or less sensitive to the effects of POPs. The following is a short, general summary of results from laboratory studies as well as results from field studies where POPs have been quantified and concentrations have been correlated with biological and toxicological effects.

A wide range of effects is seen after exposure to POPs. Some of these types of effects are currently being used as biological markers for POP exposure. These include, among other things, effects on reproduction, development (including the brain), cytochrome P450-dependent enzymes, the immune system, the adrenals, the thyroid gland, thyroid hormone levels, vitamin A levels, formation of DNA adducts, peroxisome proliferation, and gap junction intercellular communication. Almost all POPs considered in this report also cause visible changes in the liver, including hypertrophy, lesions, and in some cases, tumors.

POPs can cause short-term acute effects when administered in high doses as well as long-term chronic effects at lower doses. In the Arctic, the major concern is long-term chronic exposures as organisms are exposed to POPs over their entire lifetimes. In this context, the major effects of concern are those that may affect reproduction and survival at the individual and population level. Effects at the individual, population or ecosystem level, however, come at a late stage of exposure. It would be more useful to have earlier warning of exposure to POPs and, therefore, biological marker systems based on subtle, low-dose effects are being used or developed. Most biological markers measure effects at the molecular, cellular or organ level; however, it is still not established what these changes might mean at the individual or population level.

3.2.1. Reproduction and development

POPs have a number of effects on the ability of organisms to reproduce and develop normally (for reviews, see Peterson *et al.,* 1993; Bosveld and van den Berg, 1994; Barron *et al.,* 1995; Brouwer *et al.,* 1995). Exposure to some POPs may cause embryo- and fetotoxicity, decreased offspring survival, abnormalities in the estrus cycle and sex hormone levels, reduced sperm production, reduced litter sizes, and even total reproductive failure in mammals. In birds, some POPs cause decreased egg production, retarded egg production, increased embryo mortality, eggshell thinning, embryonic deformities, growth retardation, and reduced egg hatchability, as well as detrimental effects on parental behavior. In fish, some POPs cause decreased egg and larval survival, reduced sexual maturation, and reduced gonad size. Other effects of POPs on organisms may include structural malformations, neurotoxic effects, and neurological and behavioral changes in offspring. Behavioral changes also occur in adult animals, including changes in mating behavior.

Some POPs act as hormones or interfere with endocrine systems and are therefore called endocrine or hormone disruptors (for reviews, see Vos *et al*., 2000; Damstra *et al*., 2002). The reproductive effects of embryonic or fetal exposure to these disruptive compounds may only become obvious at later developmental stages or at sexual maturity. The estrogenic and antiestrogenic effects of POPs are the best studied of these effects. Endocrine disruption is also implicated in thyroid and immune system effects, which are treated in Sections 3.2.3 and 3.2.4. POPs may also function as androgens or antiandrogens. Estrogens and androgens are important in the normal sexual differentiation of developing organisms. A number of biomarkers have now been developed for testing the estrogenicity of POPs.

Studies have shown that there is a critical phase in neonatal mouse brain development when the brain is particularly susceptible to effects of low-dose exposure to toxic substances such as PCB, DDT, pyrethroids, organophosphates, paraquat, and nicotine (Eriksson,

1997). This critical phase is known as the 'brain growth spurt' (BGS) and disruption leads to persistent disruption in adult brain function. The BGS occurs at different time points in different mammalian species (Davison and Dobbing, 1968). In rats and mice, it occurs in the first 3-4 weeks of life (neonatal period) whereas in humans, it occurs during the third trimester of pregnancy and throughout the first two years of life. Some POPs, most notably the non-dioxin-like PCBs, may act as neurotoxins by decreasing dopamine content in the brain and altering calcium homeostasis (for a review, see Tilson and Kodavanti, 1998).

3.2.2. Cytochrome P450 system and other xenobiotic metabolizing enzyme systems

The most developed of the biological markers is the study of cytochrome P450-dependent liver enzymes (e.g., Förlin *et al.,* 1994; Jensen and Hahn, 2001; Kim and Hahn, 2002). Exposure to OCs and some PAHs induces liver cytochrome P450-dependent enzymes (CYP) known as mixed function oxidases (MFO), which metabolize xenobiotics and endogenous substances (Nebert and Gonzalez, 1987). Exposure to high concentrations of MFO-inducing POPs can affect the metabolism of endogenous substrates, such as steroid hormones, leading to disturbances in critical biological functions (Kupfer and Bulger, 1976). Other methods that can be used to study CYP forms include measuring CYP messenger ribonucleic acid (mRNA), DNA, and protein levels. Protein levels are determined using electrophoresis combined with immunoblotting, which requires the availability of antibodies for each CYP form to be identified.

There are several gene families of cytochrome P450 in vertebrates (Nelson *et al.,* 1993; 1996) and those most relevant for the metabolism of POPs are the CYP 1A, 2B, and 3A gene families. CYP1A forms are induced primarily by planar aromatic hydrocarbons such as PAHs, PCDD/Fs, non-*ortho* and some mono-*ortho* PCBs. Induction of CYP1A is mediated by the aryl hydrocarbon (Ah) receptor, a cytosolic receptor found in all vertebrates studied so far, and for which the most potent ligand is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The activated ligand-receptor complex triggers the genetic expression and production of a number of proteins including CYP1A. Induction is often measured as increases in activity of several enzymes including ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) as well as by caffeine demethylation. The efficacy of CYP1A to metabolize POPs that induce these enzymes appears to vary considerably among taxa.

CYP2B forms are induced in mammals by another class of substances, typified by phenobarbital (PB) and measured in laboratory rodents as aminopyrine N-demethylase (APND) activity, aldrin epoxidase (AE), and pentoxyresorufin-*O*-dealkylase (PROD), for example. Considerable caution is needed in interpretation of these activities in wild mammals. For example, PROD activity was shown to be more highly correlated with CYP1A than CYP2B induction in polar bears (Letcher *et al*., 1996). Birds possess one or more CYP enzymes that have an activity profile very similar to CYP2B in mammals, especially as judged by the ability to metabolize specific PCB structures (Norstrom, 1988). This enzyme may be in the CYP2 family, but there is no immunochemically recognizable CYP2B in birds. Substances that induce CYP2B are DDT, chlordane, aldrin, endrin, di- to tetra-*ortho* PCBs, and 3-$MeSO_2$-PCB. Mono-*ortho* PCB congeners and technical PCBs are mixed type inducers inducing both CYP1A and 2B. There is strong correlative evidence that CYP2B is induced by a combination of PCBs and chlordanes in polar bears (Letcher *et al*., 1996).

CYP3A forms are among the most versatile iso-enzymes with low substrate specificity. Substances that induce CYP3A include carcinogens, pesticides, some drugs, and steroid hormones such as testosterone. Induction is often measured as the formation of hydroxylated metabolites (e.g., 6-β hydroxylation) of testosterone. Recently, CYP3A has been found to be involved in the metabolism of several toxaphene congeners in harbour (*Phoca vitulina*) and grey seals (van Hezik *et al.,* 2001) and implicated in toxaphene metabolism in harp and ringed seals (Wolkers *et al.,* 1998b; 2000). A CYP3A form that is immunologically cross-reactive with anti-rat CYP3A1 was induced in chickens by PB (Ourlin *et al*., 2000). CYP3A forms may be involved in the metabolism of some POPs in birds.

In the previous AMAP assessment report, de March *et al.* (1998) reviewed species differences in the cytochrome P450 system, particularly with reference to the Arctic situation. At that time, the P450 system had been characterized in a number of Arctic species including harp and hooded seals, polar bears, harbour porpoise (*Phocoena phocoena*), beluga (*Delphinapterus leucas*), short-finned pilot whale (*Globicephala macrorhynchus*), and minke whale (*Balaenoptera acutorostrata*). For terrestrial mammals, they generally have two CYP1A isoforms known as 1A1 and 1A2 as well as functioning CYP2B forms and thus, have a high capacity for metabolizing both groups of POPs.

Harp, grey and hooded seals, and harbour porpoise, like other mammals, seem to have functional CYP1A1 and 1A2 and thus, a higher capacity to metabolize planar compounds, but have weak CYP2B activity and thus, a reduced ability to metabolize xenobiotics that are substrates for these particular MFOs (Boon *et al.,* 1992; Goksøyr *et al.,* 1992; Goksøyr, 1995a). Seals seem to have more CYP2B activity than whales (Goksøyr, 1995a). Polar bears have functional CYP1A1, 2B1, 3A1, and epoxide hydrolase (Bandiera *et al.,* 1995), and have a high metabolic capacity, particularly for PCB and DDT (Norstrom and Muir, 1994).

Previous studies have shown that fish seem to lack CYP2B and have only one version of CYP1A (Nebert *et al.,* 1989; Stegeman, 1989; Stegeman and Hahn, 1994; Goksøyr, 1995b), and thus have a low POP metabolizing capacity. However, one exception to this has been found recently. Deepwater sculpin (*Myxocephalus thompsoni*) from the Great Lakes, have been found to metabolize PCBs and form 3- and 4-$MeSO_2$-PCB metabolites, indicating that they have CYP2B-like activity (Stapleton *et al.,* 2001). With regard to birds, cytochromes P450 1A1 and 1A2 are found in this group of animals (Livingstone and Stegeman, 1989).

Since 1996, new studies have further characterized the cytochrome P450 system in Arctic species. CYP1A is present in Arctic char and is active even at low temperatures (Wolkers *et al.,* 1996; 1998c; Jørgensen and Wolkers, 1999). Recent studies in glaucous gull (*Larus hyperboreus*) have shown some EROD activity implying the presence of CYP1A (Henriksen *et al.,* 1998a; 2000). Ringed seals have been found to have CYP1A and 3A activities, and possibly CYP2B activity (Mattson *et al.,* 1998; Wolkers *et al.,* 1998a; 1998b; Hyyti *et al.,* 2001; Nyman *et al*., 2001). Besides CYP1A, both grey and ringed seals may have active CYP1B1 (Nyman *et al.,* 2000), which has previously only been found in human, rat and mouse tissues (Nelson *et al.,* 1996). CYP1B1 metabolizes PAHs, often initiating carcinogenesis (Shimada *et al*., 1996; Baron *et al.,* 1998; Kim *et al*., 1998a). Harp seals have been found to have active CYP3A (Wolkers *et al.,* 1999; 2000). Steller sea lions have been found to have CYP1A1 and 1A2, whereas minke whales only have CYP1A1 (Teramitsu *et al.,* 2000). Beluga and pilot whales (*Globicephala melas*) both have CYP1A1 activity and show the presence of CYP2B, though it is not clear if this is active (White *et al.,* 2000).

Organisms lacking functional CYP1A, 2B or 3A will not be able to eliminate the POPs metabolized by these enzymes, leading to their bioaccumulation. This is particularly the case for fish, making them amplifiers of many POPs in food webs. The presence of functional cytochrome P450 enzymes means that POPs may be metabolized and eliminated; metabolized to lipophilic and toxic metabolites; metabolized to hydrophilic metabolites that bind to proteins and are retained; and/or, that POP exposure may lead to cytochrome P450 enzyme induction, increasing the amounts of metabolic enzymes present. Those organisms that cannot eliminate POPs may accumulate parent compound concentrations high enough to cause effects. Those organisms that metabolize POPs to lipophilic and toxic metabolites, and/or hydrophilic and toxic metabolites that are retained, may instead accumulate these metabolites to high enough concentrations to cause effects.

3.2.3. Immunological effects

Many POPs disrupt both humoral and cell-mediated immune responses of the specific (acquired) arm of the immune system, as well as causing effects on the non-specific (innate) arm. As a result, the resistance to infectious agents may be reduced. Humoral-mediated immunity involves the body's ability to recognize foreign substances (helper T-cells) and mount a response by stimulating the production of antibodies (B-cells). Cell-mediated immunity is involved in delayed hypersensitivity reactions (e.g., skin reactions to allergens) and the production of cytotoxic T-cells against tumors and viruses. Natural killer cells are involved in the non-specific response (i.e., absence of memory) and are a first line of defense against virus-infected cells and tumors. Most POPs cause multiple effects on the immune system.

Some OCs (PCDD/Fs, some PCBs) as well as TBT have direct effects on the thymus, causing atrophy of this lymphoid organ responsible for the maturation of T-cells. The most insidious effect of POPs on the immune system is to decrease an organism's resistance to infection or cancer. Immunosuppressive effects of POPs have often been studied as: reduced antibody production when exposed to a foreign antigen (e.g., suppression of the anti-sheep red blood cell plaque-forming response); decreased delayed-type hypersensitivity; decreased natural killer cell activity; and, decreased resistance to pathogens (e.g., viral infections) (Vos and Luster, 1989; Tryphonas, 1994; Wong *et al.,* 1992). All of these have been used as indicators of immunosuppressive effects in laboratory and wild animals.

Immunosuppressive effects may be one of the most sensitive and environmentally relevant effects of POPs (Vos and Luster, 1989). For example, immunosuppression has been measured in harbour seals fed Baltic fish in semi-field experiments and was found to correlate with levels of PCDD/F and planar PCBs expressed as toxic equivalents (TEQs) (de Swart *et al.,* 1995; Ross *et al.,* 1995; 1996a). Immunosuppression is also suspected to be the cause of an increasing prevalence of moderate to severe intestinal ulcers in Baltic grey seals (Bergman, 1999). Indications of immunosuppression have also been found in some Arctic species including polar bears, northern fur seals, and glaucous gulls (Chapter 6).

3.2.4. Thyroid and retinol effects

Thyroid hormones control metabolism and growth, and are essential for normal reproduction. They are also important for the development of normal brain functions during fetal development (Morse *et al.,* 1993; Morse, 1995). The thyroid gland produces predominantly T4 (thyroxine), which is transported in plasma to target tissues by the transport protein TTR. Once delivered, T4 is deiodinated by T4-monodeiodinase to triiodothyronine (T3), which is the active hormone. Some POP effects on the thyroid may be related to the ability of some POP phenolic metabolites, such as hydroxylated PCBs, hydroxylated PBDEs, pentachlorophenol and 4-hydroxy-heptachlorostyrene, to attach to the binding sites on the transthyretin-retinol-binding protein complex (TTR-RBP) in plasma, thereby disrupting the normal transport of thyroid hormones T3 and T4 as well as vitamin A (retinol) to their target tissues (Rolland, 2000; Simms and Ross, 2000). POPs may also interfere with the enzymes that control thyroid hormone metabolism such as uridine diphosphate glucoronosyl transferase (UDPGT), which is involved in glucuronidation and subsequent excretion of T4 (Brouwer *et al*., 1998). Recently, TTR has been identified in polar bear plasma (Sandau *et al*., 2000) and in harbour seals (Simms and Ross, 2000).

The structural requirements for binding of a POP metabolite to TTR are hydroxy-substitution in *para-* or *meta*-positions of one or both phenyl rings with adjacent halogen unsubstituted sites (Lans, 1995). X-ray diffraction studies have shown that several phenolic organohalogen compounds bind with the hydroxy group in the central channel of the TTR molecule (Ghosh *et al*., 2000). Disruption of normal thyroid hormone transport leads to lowered plasma levels of T3 and T4, which in turn may initiate an increased release of thyroid stimulating hormone (TSH) to stimulate the thyroid gland to secrete more T3 and T4. This disruption of the feedback system for thyroid hormones may lead to thyroid

hyperplasia (goiter), hypertrophy, hypothyroidism, disruptions of metabolism and possibly a tendency to develop thyroid tumors. Another effect may also be related to the ability of some POPs, such as PCBs, to induce the production of liver enzymes involved in the breakdown of thyroid hormones. This results in reduced amounts of thyroid hormones circulating in the plasma.

Imbalance in vitamin A (retinol and its esters) status can cause immunosuppression, susceptibility to cancers, skin lesions, as well as disruption of reproduction, growth, and development. Some POPs (particularly PCDDs and PCBs) affect vitamin A metabolism and transport. Biomarkers used for thyroid and retinol effects include measuring plasma levels of free and bound T3 and T4, TSH as well as vitamin A levels.

3.2.5. Mutagenic and carcinogenic effects

Current research supports a two-stage cancer model characterized by a primary mutagenic event (initiation) followed by a long latency period and second event (promotion) that leads to tumor growth. Peroxisomes are cellular organelles mainly found in the liver and kidney. They contain peroxisomal enzymes and are essential for lipid metabolism, cellular respiration and gluconeogenesis among other things (for a review, see Youssef and Badr, 1998). A number of POPs, including perfluorinated compounds such as PFOS and PFOA, are potent peroxisome proliferators. Increased oxidative stress by peroxisome proliferation is thought to be one possible mechanism for tumor promotion (Cattley and Preston, 1995). Another mechanism may be down-regulation of gap junctional intercellular communication (GJIC), which has been linked to tumor-promoting properties of many carcinogens (Trosko and Ruch, 1998). Gap junctions are protein channels that allow for the transport of substances between cells. This communication is necessary for normal growth and function and, if inhibited, may lead to tumor promotion. Various PCBs, DDT, dieldrin, toxaphene and brominated biphenyls have been shown to inhibit GJIC in human breast epithelial cells (Kang *et al*., 1996) and methyl sulfone PCBs seem particularly potent (Kato *et al*., 1998). GJIC is measured using a bioassay where a monolayer of cells is scraped after exposure to a contaminant, then exposed to a dye and dye migration into the cells is quantified (Upham *et al*., 1997).

High doses of planar aromatic hydrocarbons induce oxidative stress possibly through induction of CYP enzymes (Toborek *et al*., 1995; Park *et al*., 1996; Hennig *et al*., 1999; Schlezinger *et al*., 1999; Slezak *et al.,* 1999; Slim *et al*., 1999). This is manifested by increased production of reactive oxygen species, lipid peroxidation and DNA damage.

Several PAHs, such as B[a]P and DMBA, are well known mutagens and both initiate and promote tumors. Most of the other POPs dealt with in this assessment are not mutagenic, but many are strong tumor promoters. Several POPs are associated with increased tumor prevalence found in highly exposed wildlife from areas outside of the Arctic, including fish (Myers *et al*., 1998) and beluga from the St. Lawrence Estuary (De Guise *et al*., 1994; Martineau *et al*., 1985).

Methods for measuring mutagenicity include the measurement of DNA adducts since there is a positive correlation between a chemical's carcinogenic potency and the extent that it binds to DNA (Kriek *et al*., 1998). Correlations have also been shown between the level of exposure to PAHs and the amount of adducts formed (French *et al*., 1996; Shugart and Theodorakis, 1998; Wirgin and Waldman, 1998). Other standard methods include the formation of micronuclei, sister chromatid exchange, chromosome aberration assays in peripheral blood lymphocytes, as well as the Ames test for single nucleotide mutations.

3.2.6. Effects of mixtures

In the previous AMAP assessment report, the additive, antagonistic and synergistic effects of mixtures were discussed (de March *et al.,* 1998). Some recent studies indicate that certain POPs singly have little or no endocrine disrupting effects but when tested as mixtures, endocrine disruption occurs, indicating synergism. For example, chlordane, dieldrin and toxaphene singly showed no ability to compete with estradiol when tested against alligator and human estrogen receptors (Arnold *et al.,* 1997; Vonier *et al.,* 1996). However, when tested as a mixture, the combination inhibited estradiol binding by 20-40%. A mixture of 15 POPs mimicking the composition in ringed seal blubber was found to disturb *in vitro* porcine oocyte maturation and development at concentrations comparable to highly exposed humans or mammals in the Arctic (Campagna *et al*., 2001).

3.3. Effects of specific POPs

Because the previous AMAP assessment report contained thorough reviews of the toxicology of legacy OCs and their metabolites (PCDDs, PCDFs, PCBs, $MeSO_2$-PCBs, aldrin, dieldrin, chlordanes, DDT, $MeSO_2$-DDE, HCB, HCHs, mirex, toxaphene), only those compounds where substantial new information has become available since 1996 are covered in this report. Emphasis has been placed on updates of these few compounds, plus the toxicology of new compounds that have been found in the Arctic. The following descriptions of the toxicology of different POPs are short reviews and not meant to be comprehensive. They mainly cover chronic effects and effects that are relevant to the Arctic discussion. Results for controlled studies in laboratory animal species are discussed first. Where done, controlled studies using wild animal species under laboratory conditions are then presented. If field studies outside of the Arctic have been performed, these are then presented. Finally, studies correlating specific effects with contaminant concentrations found from field studies of wild species, in areas with known high burdens of contaminants such as the Baltic Sea and the Great Lakes, are discussed. This last type of study has inherent problems, as it is never possible to state that the contaminant measured is the cause of the effect, since there may be other contaminants not measured that co-vary with the one measured. Field studies and correlation studies of Arctic species are discussed under Section 6. An overview of toxic effects of the POPs discussed in this report is given in Table 3.1.

Table 3·1. Overview of toxic properties of various POPs. ▼= suppression or decrease, ▲ = induction or increase.

	Reproductive/ developmental effects	*Neurotoxic effects*	*Cytochrome P450 effects*	*Immune effects*	*Thyroid/ retinol effects*	*Cancer*	*Other*
Aldrin and dieldrin	▼ Reproduction		Induces cytochrome P450 2B	Suppresses immune system		Non-mutagenic. Increased liver tumors	
Chlordanes	▼ Reproduction		Induces cytochrome P450 2B	Suppresses immune system		Non-mutagenic tumor promoter	
DDT and metabolites	Egg-shell thinning in bird eggs. ▼ Reproduction		Induces cytochrome P450 2B	Suppresses immune system	▼ Thyroid weight		Adrenal cortex hyperplasia
HCBz	Fetotoxic. Teratogenic. ▼ Reproduction		Induces cytochrome P450 1A and 2B	Suppresses immune system	▼ T3 and T4 ▲ TSH ▲ Thyroid weight	Non-mutagenic tumor promoter	▲ Porphyria
α-HCH	No information		Induces cytochrome P450 2B			Non-mutagenic tumor promoter	
β-HCH	Estrogenic		Induces cytochrome P450 2B	Suppresses immune system	▲ Thyroid weight	Non-mutagenic tumor promoter	
γ-HCH (lindane)	Estrogenic and antiestrogenic. ▼ Reproduction		Induces cytochrome P450 1A and 2B			Non-mutagenic tumor promoter	
Mirex	▼ Reproduction		Induces cytochrome P450 2B	Suppresses immune system		Non-mutagenic. Induces tumors	
Toxaphenes	Fetotoxic. ▼ Reproduction		Induces cytochrome P450 1A, 2B and 3A	Suppresses immune system	▲ Thyroid-weight ▲ TSH	Mutagenic, potent carcinogen. Inhibits GJIC	▲ Bone brittleness in fish. Adrenal hypertrophy
Endosulfan	Fetotoxic. ▼ Reproduction		Induces cytochrome P450 1A and 2B	Suppresses immune system		Non-mutagenic	
PCDD/Fs and nPCBs and metabolites	Fetotoxic. Deformities. ▼ Reproduction	Permanent changes in learning, behavior, memory (nPCB)	Induces cytochrome P450 1A	Thymic atrophy. Suppresses immune system	▼ T3 and T4 ▼ Vitamin A	Non-mutagenic tumor promoters. Affects GJIC	▲ Porphyria
Other PCBs	Fetotoxic. Deformities. ▼ Reproduction	Permanent changes in learning, behavior, memory. Decreased dopamine	Induces cytochrome P450 2B	Suppresses immune system	▼ T3 and T4 ▼ Vitamin A	Non-mutagenic tumor promoters. Affects GJIC	▲ Porphyria Hyperadrenocortism
SCCPs	Fetotoxic Deformities. ▼ Reproduction	▼ Motor performance	Induces cytochrome P450 1A	No information	▼ T4 ▲ TSH	Non-mutagenic. ▲ Peroxisome proliferation. Inhibits GJIC	
PCNs	Embryotoxic. ▼ Reproduction		Induces cytochrome P450 1A				
Octachlorostyrene and metabolites			Induces cytochrome P450 1A and 2B		Binds to TTR *in vitro*		
PBDEs	Estrogenic and antiestrogenic	Permanent changes in learning, behavior, memory	Induces cytochrome P450 1A and 2B	Suppresses immune system	▼ T4 ▼ Vitamin A	Non-mutagenic	
PFOS/PFOA	▼ Reproduction					Non-mutagenic, tumor promoter ▲ Peroxisome proliferation. Inhibits GJIC	
TBT and metabolites	Imposex in invertebrates. Deformities. ▼ Reproduction		Inhibits liver cytochrome P450 1A, 2B, and 3A	Suppresses immune system		May be carcinogenic	

3.3.1. Halogenated industrial chemicals and by-products

3.3.1.1. Update on PCDDs, PCDFs, and PCBs, including PCB metabolites

The 2,3,7,8-PCDD/Fs, as well as PCBs substituted in the 3,3',4,4'-positions with no (non-*ortho* PCB (nPCB)) or one *ortho* chlorine (mono-*ortho* PCB) are among the most toxic POPs. The most toxic and best studied of these planar compounds is TCDD. These substances exert their toxic effects via a common mechanism that requires binding to the Ah receptor. They produce essentially the same spectrum of toxic effects in treated animals as TCDD, differing only in their potencies. Except in some bird species, all are less potent than TCDD. The non-*ortho* PCBs are more toxic than the mono-*ortho* congeners. A thorough review of the effects of PCDDs, PCDFs, and PCBs was presented in the previous AMAP assessment report (de March *et al*., 1998). The following is an update on relevant effects studies published since 1996.

Reproductive and developmental effects

Competitive-binding studies using estrogen receptors from humans, green anole (*Anolis carolinensis*), and rainbow trout (*Salmo gairdnerii*) showed that chlorobiphenyls (CBs) 104, 184, and 188 were significant competitors in all three systems and thus have estrogen-like activity (Matthews and Zacharewski, 2000). In the rainbow trout assay, CBs 41, 51, 91, 115, 143, and 173 were also found to compete to some extent. None of these congeners are, however, major components in commercial PCB mixtures, and some are not present at all. The findings are therefore of small relevance to the environment. Hydroxy-PCBs that are found in blood have been shown to be weakly anti-estrogenic in the MCF-7 human breast cancer cell line and in HeLa cells (Kramer *et al.,* 1997; Moore *et al.,* 1997). Andersson *et al*. (1999) found that four hydroxy-PCBs significantly induced vitellogenin synthesis in rainbow trout hepatocytes and induced proliferation of MCF-7 cells, both indicative of the capabilities of these substances to bind to the estrogen receptor and elicit a response. Also shown, was the induced proliferation of MCF-7 cells by CBs 104 and 188.

Experiments have been performed exposing neonatal mice to a single oral dose of specific CB congeners at a critical time point during the brain growth spurt and studying a range of neurobehavioral effects (Eriksson *et al.,* 1991; Eriksson and Fredriksson, 1996a; 1996b; 1998). Permanent changes in spontaneous behavior were seen after neonatal exposure to *ortho*-substituted PCBs (2,4,4'-TrCB (CB28) at 14 µmol (3.6 mg)/kg body weight; 2,2',5,5'-TeCB (CB52) at 14 µmol (4.1 mg)/kg body weight; 2,2',4,4',5,5'-HxCB (CB153) at 14 µmol (5.1 mg)/kg body weight); non-*ortho* PCBs (3,3',4,4-TeCB (CB77) at 14 µmol (4.1 mg)/kg body weight; 3,3',4,4',5-PeCB (CB126) at 0.14 µmol (0.046 mg)/kg body weight; and, 3,3',4,4',5,5'-HxCB (CB169) at 1.4 µmol (0.51 mg)/kg body weight). The effects worsened with age. As well, learning and memory in adult mice were affected by CB52 and CB153 at the same doses, CB126 at 1.4 µmol (0.46 mg)/kg body weight, and CB169 at 14 µmol (5.1 mg)/kg body weight.

In previous studies reviewed in the AMAP assessment report, Holene *et al*. (1995; 1998) showed significant behavioral alterations in rat offspring after pre- and post-natal exposure (including lactation exposure in males) to several specific CB congeners (CBs 118, 126, 153). In a recent study (Holene *et al.,* 1999), female rats were exposed to CB153 through mother's milk. The females showed a significant sex-specific behavioral response, being less sensitive than males studied previously, since only deficient acquisition of time discrimination was seen.

Repeated exposure to Aroclors caused decreases in brain dopamine and the bioaccumulation of several *ortho*-substituted CBs in the brain of rats and non-human primates (Seegal *et al*., 1986; 1991a; 1991b; Shain *et al*., 1986; 1991; Seegal and Schantz, 1994). Kodavanti *et al*. (1995; 1996) have since been able to show that the *ortho*-substituted, non-dioxin-like PCB congeners are associated with neurotoxicity. The *ortho*-PCBs were found to disrupt intracellular signal transduction in cells from the cerebellum. Mariussen *et al*. (1999) tested 14 PCBs *in vitro* for their ability to competitively inhibit dopamine uptake into synaptic vesicles and found that only *ortho*-PCB congeners were active. EC_{50}s (the concentration affecting 50% of the animals) ranged from 2.3 to 5.6 µg/g (7-30 µM) for single congeners. In non-human primates, brain concentrations of 2 to 5 µg/g of ΣPCB were associated with decreased dopamine concentrations (Seegal *et al*., 1990). In a study of 20 PCB congeners, inhibition of dopamine uptake in synaptic vesicles was found to be most potent for CBs 41, 91, 112, and 143 (all *ortho*-substituted) and no inhibition was seen with the non-*ortho* CBs tested (Andersson, 2000).

Aroclor 1254 has been found to affect bone development in rats (Andrews, 1989) and recently, CB126 (3,3',4,4',5-PeCB) has been shown to cause extensive alterations in the long bones of rats, including decreases in density and strength (Lind *et al*., 1999; Lind, 2000). TCDD also affects bone structure in rats in a similar manner (Jämsä *et al*., 2001) and has also been found to impair their molar tooth development (Kattainen *et al*., 2001; Lukinmaa *et al*., 2001). A synthetic mixture of 16 $MeSO_2$-PCBs, prepared in the same relative concentrations as found in the blubber of Baltic grey seals, was fed to female mink (*Mustela vison*) for one year and reproductive outcome was studied (Lund *et al*., 1999). Compared to controls, the treated mink had significantly increased litter size, but lower birth weights and reduced kit survival. *In vitro* studies of liver showed increased breakdown of progesterone in the treated mink. The muscle concentrations found in the exposed females and their kits were 18 000 ng/g lw and 21 000 ng/g lw, indicating considerable carry-over from dams to kits.

In mink, continuous exposure to 250 ng PCB/g food caused delayed onset of estrus and lowered whelping rates (Restum *et al*., 1998). PCB exposure was from a diet made using Saginaw Bay carp as the PCB source. Exposure to 500 ng PCB/g food led to increased litter mortality and reduced kit body weights. Short-term parental exposure reduced kit survival of subsequent generations of mink conceived months after the parents were placed on PCB-free feed.

Kestrels (*Falco sparverius*) were exposed to PCBs *in ovo* via parents that were fed a diet containing a mixture of technical PCBs (7 mg/kg body weight/day) through

breeding and hatching (Fernie *et al*., 2001). This diet led to an exposure of 34 µg PCB/g whole egg ww. These second-generation kestrels were paired with unexposed, experienced kestrels and compared with non-exposed controls. *In ovo* PCB exposure was found to suppress egg laying in 25% of exposed females, delay clutch initiation and lead to smaller clutch sizes for both exposed sexes. Exposed kestrels also had reduced fledgling success and higher incidence of complete brood mortality. Greater effects were seen in PCB-exposed females than males.

A mixture of 20 PCB congeners administered to zebrafish (*Brachydanio rerio*) via food caused reproductive disturbances (Örn *et al*., 1998). Egg production and offspring survival time were reduced. CBs 60, 104, and a hydroxy-PCB were highly embryotoxic in zebrafish oocytes exposed via maternal transfer (Westerlund *et al*., 2000). A significant negative correlation was found between ΣPCB levels above 20 ng/g ww (as Aroclor 1260 in liver) and baculum (penis bone) length in wild juvenile mink (Harding *et al*., 1999).

Aroclor 1242 caused significant sex reversal in red-eared sliders (*Trachemys scripta elegans*), a species of turtle, overriding male-producing temperature levels to result in female hatchlings, indicating that this PCB mixture has estrogenic effects (Willingham and Crews, 1999).

Later egg laying, prolonged incubation, and smaller eggs and chicks were correlated with higher yolk sac PCDD/F concentrations in common tern chicks (*Sterna hirundo*) (Murk *et al*., 1996). Toxic effects in colonial fish-eating birds in the Great Lakes area of North America were correlated with the concentrations of PCDDs/PCDFs and non-*ortho* PCBs found in the different bird species studied (reviewed in Gilbertson *et al.,* 1991; Giesy *et al.,* 1994a; 1994b). The effects in birds include reduced egg hatching, embryotoxicity, deformities such as crossed bills and clump feet, and impaired parental behavior (Hoffman *et al.,* 1987; Kubiak *et al.,* 1989; Tillitt *et al.,* 1989; 1991; 1992; 1993; Yamashita *et al.,* 1993).

Cytochrome P450-dependent monooxygenases

Rats and mice exposed to either Aroclor 1254 or CB105 had induced cytochrome P450 activity measured as EROD, methoxy-*O*-resorufin deethylase (MROD) and PROD activity, with rats being more sensitive than mice (Hallgren *et al*., 2001).

In the study of 16 $MeSO_2$-PCBs fed to mink, discussed previously, an 11-fold induction of PROD activity was seen in the exposed adult females and a five-fold increase was seen in their five-week-old kits (Lund *et al*., 1999). This is in accordance with previous research showing high CYP2B induction for $MeSO_2$-PCBs in rats (Kato *et al*., 1995a; 1995b; 1997; 1999).

Mink fed a diet with varying PCB doses made from clean fish or contaminated carp from Saginaw Bay, Lake Huron, had dose-dependent induction of cytochrome P450 activity (Shipp *et al*., 1998). Young mink were more sensitive than older mink.

Adult male mallard ducks (*Anas platyrhynchos*) dosed orally with Aroclor 1254 had induced cytochrome P450 activity as measured by elevated EROD and PROD activity (Fowles *et al*., 1997).

In an experimental study, Arctic char were divided into four groups, PCB-exposed and fed, PCB-exposed and starved, non-exposed and fed, and non-exposed and starved (Jørgensen *et al*., 1999). The PCB-exposed fish were fed a single oral dose of 1 µg Aroclor 1260/g body weight. Increased EROD activity was found in the PCB-exposed and starved group, which also had the highest PCB concentrations and exhibited fin erosion. The threshold for EROD effects in Arctic char was found to be 1 µg PCB/g ww in liver.

Induction of hepatic CYP1A1 activity was found to be correlated with PCDD/F concentrations in common tern chicks (Murk *et al*., 1996). EROD induction was found to correlate with ΣPCB and DDT levels in a comparison of ringed and grey seals from the Baltic Sea and from reference sites (Nyman, 2000).

Immunosuppression

Beluga leukocytes and splenocytes were exposed to several POPs *in vitro*, and CB138 was found to significantly reduce splenocyte proliferative responses (De Guise *et al*., 1998). No effects were seen with CBs 153, 180, 169 or TCDD.

Previously, a diet of Baltic Sea herring (*Clupea harengus*) fed to captive harbour seals was found to cause immunosuppression, which was associated with the content of PCDD/PCDF and dioxin-like PCBs (de Swart *et al*., 1994; 1995; Ross *et al*., 1995; 1996a). In a laboratory study, using rats as surrogates for harbour seals, pregnant rats were administered extracts from the same diets of Atlantic or Baltic Sea herring as were given to harbour seals, or a positive control of Atlantic herring oil spiked with TCDD, on a daily basis for 41 days (Ross *et al*., 1997). Immune function was assessed in the offspring and found to be most suppressed in the TCDD-spiked group (reduced thymus weight, thymocyte and splenocyte proliferative response, natural killer cell and specific antibody responses). Similar types of immunosuppression were seen in the Baltic herring group but were less pronounced. The daily intakes for the pregnant rats were 0.3 pg TEQ/g body weight for the cleaner Atlantic herring oil, 2.1 pg TEQ/g body weight for the Baltic Sea herring oil and 134 pg TEQ/g body weight for the spiked oil.

Two juvenile harp seals were exposed experimentally to increasing doses of selected PCB congeners (0.4, 2.0, 4.0, 20.0, and 40.0 mg/day, one week per dose), while two more seals acted as controls (Lohman *et al*., 2002a). The *in vitro* release of tumor necrosis factor alpha (TNF-α) from isolated monocytes stimulated with *Escherichia coli* (*E. coli*) lipopolysaccharides and β-1,3-glucan were assayed during the treatment period and during a subsequent 30-day fasting period. Changes in the adrenal gland were also studied (Lohman *et al*., 2002b). No differences were seen between the two groups until after 30 days of fasting. Release of TNF-α was elevated in both groups, with higher levels in the controls than in the PCB-treated seals, indicating possible decrease in monocyte reaction to lipopolysaccharides. Elevated basal levels of serum cortisol and aldosterone were seen in the PCB-exposed seals, compared to the control seals. After 30 days of fasting, both groups had increased levels of cortisol and aldosterone compared to the treatment period, with levels in the PCB-treated seals exceeding those in the controls. Although this study is limited because of small sample sizes, the results may indicate

that short-term exposure to PCB may induce hyperadrenocorticism without noticeable morphological changes in the adrenal gland of seals.

No immunosuppression was seen in rats fed diets containing beluga blubber from the St. Lawrence Estuary (highly PCB contaminated), the Arctic or combinations of the two (Lapierre *et al*., 1999). Mice fed diets based on varying amounts of beluga blubber from the St. Lawrence Estuary and the Arctic, providing different PCB exposures, showed some indications of immunosuppression but no difference was seen between the treatment groups (Fournier *et al*., 2000).

Chicken embryos exposed to CB126 *in ovo* had decreased thymus mass, decreased live T-cell numbers in the thymus, declines in some types of thymocytes, and decreased viable B-cell numbers in the bursa of Fabricius (Grasman and Whitacre, 2001).

Glaucous gull chicks with high and low dietary PCB exposure were immunized with antigen. Antibody production, lymphocyte response, mitogen-induced lymphocyte proliferation and hematology were measured after 56 days of age (Larsen *et al*., 2002d). A significantly higher lymphocyte response to phytohemagglutinin (PHA) in the high PCB group indicated general immune system stimulation. Significantly lower antibody titers in response to influenza virus immunization in the high PCB group indicated impaired ability to produce antibodies and may be associated with decreased resistance to infections.

In the study on Arctic char by Jørgensen *et al*. (1999), previously described in the section on P450-dependent monooxygenases, increased plasma cortisol levels were seen in the PCB-exposed and fed group. In a similar study, Jørgensen *et al*. (2002b; 2002c) exposed Arctic char to varying, single doses of Aroclor 1254 and held them either with or without food for five months before subjecting them to a ten-minute handling disturbance. Starved fish were given doses of 0, 1, 10 or 100 µg/g body weight and fed fish were given doses of 0 or 100 µg/g body weight. Starved control fish had elevated plasma cortisol levels compared with fed fish before handling. These basal cortisol levels were suppressed by PCBs in starved fish and were elevated in fed fish. The cortisol response to handling was suppressed by PCBs in a dose-dependent way in starved fish. Plasma glucose levels were affected in the same manner as cortisol levels. The findings indicate that stress responses in Arctic char are compromised by PCBs and the effect of fasting makes char sensitive to the effects of PCBs.

The char were also subjected to tests of disease resistance (Maule *et al*., 2002). Mortality after exposure to a disease agent was dose-related (0, 1, 10, and 100 µg/g body weight) among the starved fish, with a significant trend toward higher disease susceptibility with increasing PCB dose. No differences in mortality were seen in the two fed groups (0 and 100 µg/g body weight). However, total mortality was higher in the fed groups compared to the starved groups. The results indicate that PCB reduces immunocompetence in starved Arctic char in a dose-dependent manner, but that lean fish are also more disease-resistant than fed fish. Similarly, juvenile chinook salmon (*Oncorhynchus tshawytscha*) exposed to Aroclor 1254 had higher mortality than controls when exposed to a bacterial pathogen (Arkoosh *et al*., 2001).

Herring gull (*Larus argentatus*) and Caspian tern (*Sterna caspia*) chicks from five Great Lakes sites were assessed for immune function using the PHA skin test, a sensitive indicator of T-cell-mediated immunity in birds. Suppression of T-cell-mediated immunity was found to correlate most strongly to higher ΣPCB concentrations (Grasman *et al*., 1996; Luebke *et al*., 1997; Grasman and Fox, 2001).

Significant correlations have been found between high ΣPCB concentrations, suppressed T-cell function and increased antibody titers after immunization in Caspian terns from Lake Huron (Grasman and Fox, 2001). Significant negative correlations were found between yolk sac levels of PCDD/F, ΣPCB, non-*ortho* PCBs and TEQs, and plasma corticosterone levels in herring gull embryos from the Great Lakes (Lorenzen *et al*., 1999). The activities of phosphoenolpyruvate carboxykinase and malic enzyme, two metabolic enzymes regulated in part by corticosteroids, were also negatively correlated to yolk sac PCDD/F concentrations.

A significant association was found between ΣPCB concentrations and mortality due to infectious disease in harbour porpoises from England and Wales indicating a possible causal relationship between PCB exposure and immunosuppression (Jepson *et al*., 1999).

Thyroid and retinol effects

Until 1996, only a few hydroxy-PCB metabolites had been identified and studied. Since then, up to 30 hydroxy-PCBs have been detected in plasma of various species and 13 such metabolites have been identified (Letcher *et al.,* 2000a). Most of the identified hydroxy-PCB metabolites have the hydroxy-group attached to one of the two *para*-positions with chlorine atoms attached *ortho* to the hydroxy-group. This structural configuration mimics that of T4, the thyroid hormone, except that T4 has iodine atoms instead of chlorines. The affinities of several hydroxy-PCBs for TTR, the plasma protein that transports T4, are up to ten times greater than for the natural hormone (Brouwer *et al*., 1998; Lans *et al*., 1993; 1994). 4-OH-3,3',4',5-TeCB (CB77 metabolite) and 4-OH-CB107 reduced total plasma T4 levels in pregnant mice and in the fetuses (Sinjari *et al.,* 1998a) but the effect was less when CB77 was administered and allowed to metabolize *in vivo* (Darnerud *et al.,* 1996). 4-OH-2',3,3',4',5-PeCB (CB105 metabolite) was also found to reduce T4 levels but no effect was seen with 4-OH-2,3,3',4',5-PeCB (CB105 metabolite) (Sinjari and Darnerud, 1998). Hydroxy-PCBs also influence T4 metabolism by inhibiting sulfation, a major regulation pathway in the fetus. Inhibition of sulfation has been shown to occur *in vitro* (Schuur *et al.,* 1996; 1998; 1999). If this occurs *in vivo*, it has implications for fetal brain development (Brouwer *et al.,* 1998). Aroclor 1254 or CB105 treatment significantly reduced free and total plasma T4 levels and hepatic vitamin A in rats and mice but had no effect on levels of TSH (Hallgren *et al*., 2001).

In the study of 16 $MeSO_2$-PCBs fed to mink, discussed previously, plasma concentrations of total T3 and T4 were reduced in the exposed dams (Lund *et al*., 1999). The authors speculate that the $MeSO_2$-PCBs decrease the total T4 concentration via enzyme induction of UDPGT, which is involved in glucuronidation of T4.

Total and free plasma T4 concentrations increased with increasing TEQ levels in mink exposed via a diet of carp from Saginaw Bay, Lake Huron, Michigan, but total and free T3 decreased, indicating a reduction in T4-monodeiodinase activity (Heaton *et al*., 1995). In a similar study, T4 concentrations were found to be significantly higher in mink fed a fish diet containing PCB than in those not exposed to PCB (Nieminen *et al*., 2000). Rats fed a diet containing Baltic Sea herring that had previously been used in a semi-field study of immunosuppression in harbour seal (Ross *et al*., 1995), were found to have lower plasma T4 levels compared to the control group (Ross *et al*., 1996b).

Chick embryos exposed *in ovo* to Aroclor 1242 or 1254 had reduced plasma T4 concentrations and reduced hepatic monodeiodinase activity (Gould *et al*., 1999). A correlation was also seen between femur length and plasma concentrations of T3 and T4, indicating a decrease in skeletal growth due to reduced thyroid hormone levels. Adult male mallards dosed with Aroclor 1254 had significantly increased thyroid weights and decreased plasma total T3 concentrations (Fowles *et al*., 1997). Adult great blue herons (*Ardea herodias*) dosed with 2,3,7,8-TCDD had a significant increase in plasma T4 levels, but no effects were seen on plasma total T3 or the T3:T4 ratio (Janz and Bellward, 1997). White leghorn hens were fed a diet with different PCB levels derived from carp from Saginaw Bay, Lake Huron (Zile *et al*., 1997). The high PCB intake group had decreased molar ratios of retinol to retinyl palmitate in eggs.

CB77 injected intraperitoneally into brook trout (*Salvelinus fontinalis*) caused decreased plasma retinol in males (Ndayibagira *et al*., 1994) and exposure with CB77 in rainbow trout caused increased hydroxylation of retinoic acid due to induction of CYP1A enzymes (Gilbert *et al*., 1995). Oral dosing of lake trout (*Salvelinus namaycush*) with CB126 caused decreased liver retinoids (Palace and Brown, 1994).

In free-living European otter (*Lutra lutra*), a strong negative correlation was found between hepatic vitamin A levels and TEQs calculated from non- and mono-*ortho* PCBs (Murk *et al*., 1998), and animals with the higher concentrations had a higher incidence of infectious diseases. Immature northern elephant seals (*Mirounga angustirostris*) with northern elephant seal skin disease had depressed total T3 and T4 levels, depressed retinol levels, and higher concentrations of PCB (and *p,p'*-DDE) compared to unaffected controls (Beckmen *et al*., 1997). The ratio of T3 to T4 was significantly correlated to the concentration of CB169 in grey seals from the U.K. (Hall *et al*., 1998). Plasma thyroid hormone concentrations and PCBs were measured in a group of grey seal pups from the Norwegian west coast (Froan, Trondheimfjord) and compared to a more highly exposed group in the Baltic Sea (Sørmo *et al*., 2002). No relationship was found for T4, but T3 levels were lower in the more contaminated Baltic pups as compared to the Norwegian pups. Disruption of vitamin A and/or thyroid hormones related to high PCB concentrations has also been observed in other captive and free-ranging seal species (Brouwer *et al.,* 1989; de Swart *et al.,* 1994; Rolland, 2000; Simms *et al.,* 2000; Simms and Ross, 2000).

Decreased yolk sac retinoids and plasma thyroid levels, and increased ratios between plasma retinol levels and yolk sac retinyl palmitate were significantly correlated to higher yolk sac PCDD/F concentrations in common tern hatchlings from Belgium and the Netherlands (Murk *et al*., 1996). Lower plasma retinol levels in herring gull and Caspian tern chicks from several Great Lakes colonies were associated with high ΣPCB concentrations in eggs from the same colony (Grasman *et al*., 1996).

Higher PCB concentrations were found to be associated with increased metabolism of retinoic acid by cytochrome P450 enzymes, decreases in hepatic retinoid stores and an increase in developmental deformities in lake sturgeon (*Acipenser fulvescens*) from the St. Lawrence River compared to a reference site (Doyon *et al*., 1999).

Cancer

The coplanar PCBs, CB77 and 169, as well as TCDD, inhibit GJIC in mouse Hepa1c1c7 cells (de Haan *et al*., 1994). Of 20 tested PCB congeners, 14 were found to inhibit GJIC in rat liver white blood cell culture (Andersson, 2000). Most potent were CBs 51, 143, 173, and 184 (tri- and tetra-*ortho* congeners). Other active congeners included CBs 41, 60, 91, 104, 112, 115, 153, 188, 190, and 193. Inactive congeners were CBs 58, 68, 78, 99, 126, and 169.

Glaucous gull chicks from Svalbard were fed a clean (Arctic cod, hen eggs) or a contaminated (Arctic cod, gull eggs) diet (Krøkje *et al*., 2002). Chromosome aberrations and DNA adducts were measured and higher frequencies of aberrations were seen in both males and females of the exposed group. DNA adduct levels were also higher in males of the exposed group.

3.3.1.2. SCCPs/C_{10}-C_{13} polychlorinated *n*-alkanes

For reviews of the toxicology of SCCPs, see Environment Canada (1993), Willis *et al.* (1994), WHO (1996) and Tomy *et al.* (1998). There are difficulties in assessing the toxicity of SCCPs as most data have been generated using technical SCCP products, which can contain thousands of chemical compounds. This makes it difficult to study the toxicity of individual components, and effects may be due to stabilizers and impurities in the products. As well, degradation, bioaccumulation, and metabolism change the relative amounts of SCCP components found in organisms in the environment, making it difficult to assess exposure. Recently, studies examining the effects of SCCPs on fish have concluded that they have low acute toxicity and a narcotic mode-of-action (Fisk *et al*., 1999b; Cooley *et al*., 2001), although histopathological lesions were observed in the livers of exposed rainbow trout (Cooley *et al*., 2001). In general, SCCPs appear to be much less toxic than other persistent organic pollutants.

Reproductive and developmental effects

Pregnant rats were dosed orally with 0, 100, 500, and 2000 mg/kg body weight/day of a SCCP (58% Cl) on days 6 through 19 of gestation to study teratogenic effects (WHO, 1996). The high-dose group of dams had 32% mortality and decreased body weight, and there were increased incidences of post-implantation loss, fetal malformations, and decreases in viable fetuses. The

no-observed-effect-level (NOEL) for teratogenic effects was set at 500 mg/kg body weight/day. In pregnant rabbits exposed orally to 0, 10, 30, and 100 mg/kg body weight/day on gestation days 6 through 27, no effects were seen on dams or fetuses, and the no-observed-adverse-effect level (NOAEL) was set at 100 mg/kg body weight/day.

Mallard ducks were exposed to dietary concentrations of 0, 28, 166, and 1000 mg/kg of a SCCP (58% Cl) in a one-generation test (cited in Willis *et al*., 1994). No effects were seen on the adults but some eggshell thinning was seen although the authors questioned the biological significance of this. Hatchlings were fed the same diets for 14 days and those in the high-dose group showed 10% mortality.

Neurological effects

Mice exposed intravenously to single doses of 30-300 mg/kg body weight of two SCCPs (49% and 70% Cl) were studied for effects on motor performance and thermoregulation (Eriksson and Kihlström, 1985). Dose-dependent decreases in motor performance and thermoregulation were seen with increasing doses of both SCCPs. Mice exposed to 300 mg/kg body weight of the lower chlorinated SCCP showed significantly decreased motor performance 15 minutes after injection. As well, significantly decreased rectal temperature was seen in mice injected with 300 mg/kg body weight of both SCCPs after 60 minutes.

Cytochrome P450-dependent monooxygenases

Cytochrome P450 concentrations increased in rats exposed intraperitoneally to 1000 mg/kg body weight/day of two short-chain SCCPs (49% and 71% Cl) for four days (Nilsen and Toftgård, 1981). The increase was greater for the more highly chlorinated SCCP. Cytochrome P450 induction potential was studied in rats using five different SCCP formulations dosed orally at 1000 mg/kg body weight/day for four days (Nilsen *et al.,* 1981). Only the higher chlorinated short-chain formulations (59% and 71% Cl) led to increased P450 concentrations and EROD activity. A lower chlorinated short-chain (49% Cl), a medium-chain (50% Cl), and a long-chain formulation (49% Cl) had no effect.

High single oral doses (1000 mg/kg body weight) of C_{10}-C_{13} SCCP (49% Cl) caused an increase in benzo[a]pyrene hydroxylase activity in female flounder held in brackish water (Haux *et al.,* 1982). No effects were seen in females kept in seawater, males kept in brackish or seawater, or any fish exposed to a more highly chlorinated product (70% Cl). Rainbow trout exposed to two C_{12} SCCPs (56% and 69% Cl) showed no induction of P450 CYP1A as measured by EROD activity (Fisk *et al.,* 1996).

Immunosuppression

There is no information on the immunotoxicity of SCCPs.

Thyroid effects

UDPGT is produced in the liver and decreases plasma T4 levels, stimulating TSH release by the pituitary gland. Rats exposed to 1000 mg/kg body weight of two SCCPs (56% and 58% Cl) showed two-fold increases in plasma TSH levels, a 30-40% decrease in total and free T4, a two-fold increase of UDPGT activity but no effect on T3 levels (Wyatt *et al.,* 1993). Elcombe *et al.* (1994) observed a similar increase in UDPGT activity and TSH level and a decrease in T4 levels in male and female rats exposed to a SCCP (58% Cl) at similar doses to the above study. They also noted thyroid follicular cell hypertrophy.

Cancer

A C_{10}-C_{23} SCCP (70% Cl) was not found to be mutagenic in the Ames test using three different strains of *Salmonella typhimurium* (Meijer *et al.,* 1981). A SCCP (60% Cl) was found to increase hepatocellular neoplasms in both sexes of mice and rats; kidney tubular cell adenomas; adenocarcinomas and mononuclear cell leukemia in male rats; and, thyroid follicular cell neoplasms in female rats and mice (Bucher *et al.,* 1987). The rats were given repeated oral doses of 312 and 625 mg/kg body weight/day and the mice, 125 and 250 mg/kg body weight/day and their responses were followed for two years.

Peroxisomal proliferation, as measured by significantly increased peroxisomal fatty acid oxidation, was seen in rats and mice exposed to two SCCPs (56% and 58% Cl) (Wyatt *et al.,* 1993). Peroxisome proliferation was confirmed using microscopic methods in rats exposed to the same two SCCPs (Elcombe *et al.,* 1994).

SCCPs (50% and 60% Cl) have been found to be potent inhibitors of GJIC in rat liver epithelial cells, indicating that they may be tumor promoters (Kato and Kenne, 1996).

3.3.1.3. **PCNs**

PCNs are planar molecules, like PCDDs, PCDFs, and non-*ortho* PCBs, and also seem to exert their effects via the Ah receptor. Acute and chronic exposure to PCNs leads to effects similar to those seen for PCDDs, PCDFs and non-*ortho* PCBs (for reviews, see Kover, 1975; Brinkman and Reymer, 1976; Crookes and Howe, 1993; Jakobsson and Asplund, 2000). The most toxic congeners are the PeCNs and HxCNs. Based on *in vitro* studies, several PCN congeners have been assigned TCDD toxic equivalency factors (TEFs) (Hanberg *et al.,* 1990; 1991; Blankenship *et al.,* 2000; Villeneuve *et al.,* 2000).

Reproductive and developmental effects

A commercial PCN product (Halowax 1014) as well as a mixture of 1,2,3,5,6,7- and 1,2,3,4,6,7-hexachloronaphthalenes are both toxic to chick embryos (Engwall *et al*., 1993; 1994). Male and female chickens fed different doses of Halowax 1014 have been mated and egg production studied. At higher doses (20 mg/kg), egg hatchability was reduced and no eggs were produced in chickens fed the highest dose of 100 mg/kg (Pudelkiewicz *et al*., 1959).

In another study, Halowax 1014, 1013 or 1051 were nanoinjected into fertilized medaka (*Oryzias latipes*) eggs at various dose levels (0.3-30 ng/egg) and the embryos allowed to develop to adulthood and sexual maturity (Villalobos *et al.,* 2000). Early life stage and early adult life stage assessments were carried out. Halowax

1014 was found to be more toxic than Halowax 1013 and 1051. The 16-day LD_{50} (dose that kills 50% of the exposed animals) for Halowax 1014 in embryos was 4.2 ng/egg and death was caused by cardiovascular abnormalities. The lowest-observed-adverse-effect level (LOAEL) was 3.0 ng/egg with hemorrhage and yolk sac edema as the major effects. Halowax 1014 decreased the gonadosomatic index in adult females. Halowax 1013 caused high mortality at 10 ng/egg and premature hatching of embryos at all doses. Halowax 1051 was the least toxic PCN.

Cytochrome P450-dependent monooxygenases
Three-spined sticklebacks (*Gasterosteus aculeatus*) fed the commercial PCN mixture Halowax 1014 have shown a dose-related increase in EROD activity as well as lipid accumulation in the liver (Holm *et al*., 1993). Rainbow trout fry show dose-related increases in EROD activity after microinjection of Halowax 1014 at the embryo stage (Norrgren *et al*., 1993). A commercial mixture of tetra-, penta- and hexachlorinated PCN (Halowax 1014) as well as a mixture of 1,2,3,5,6,7- and 1,2,3,4,6,7-HxCNs both caused liver enzyme induction in chick and eider duck embryos (Engwall *et al*., 1993; 1994). A HpCN congener had much lower EROD induction potency.

Three *in vitro* bioassays were used to test the ability of 18 PCN congeners to induce CYP1A activity (Villeneuve *et al.,* 2000). The PLHC-1 fish hepatoma cell bioassay was fairly insensitive to PCNs but the EROD and luciferase assays using recombinant H-4-II E rat hepatoma cells were more sensitive. The HxCN congeners tested were most potent, followed by the PeCNs. The TeCNs, TrCNs, DiCNs, and MoCNs tested were less active.

Rainbow trout sac fry were treated with Halowax 1014, a mixture of 1,2,3,4,6,7- and 1,2,3,5,6,7-HxCN, or 1,2,3,4,5,6,7-HpCN injected into the yolk sac (Pesonen *et al.,* 2000). After two weeks, immunohistochemical analysis was performed for CYP1A expression and was most pronounced in the hepatocytes. Exposure of a primary cell culture of trout hepatocytes to these PCNs led to increased EROD activity and CYP1A mRNA content, with the HxCN mix being most potent followed by HpCN and then Halowax 1014.

3.3.1.4. **OCS**

Long-term dietary exposure of rats to OCS causes elevated serum cholesterol and histological changes in the thyroid, liver, and kidney of rats (Chu *et al*., 1986a).

Reproductive and developmental effects
OCS was found to have binding affinity for both the androgen and estrogen receptor in an *in vitro* assay (Satoh *et al*., 2001).

Cytochrome P450-dependent monooxygenases
OCS significantly induced CYP1A activity in mice (Smith *et al*., 1994). Long-term dietary exposure to high doses of OCS induced the CYP2B enzymes aniline hydroxylase and aminopyrine N-demethylase (APND) in male and female rats, with males being more sensitive (Chu *et al*., 1986a).

Thyroid and retinol effects
Sandau *et al*. (2000) found that 4-hydroxy-heptachlorostyrene (4-OH-HpCS), a metabolite of OCS, had a similar affinity for human TTR as T4, the native hormone. The potential of 4-OH-HpCS to bind to TTR makes it capable of disrupting thyroid hormone transport and potentially affecting circulating retinol concentrations.

3.3.1.5. **Update on PBDEs**

PBDEs are numbered according to the same system as PCBs, based on chlorination degree and placement of the chlorines on the two aromatic rings. Long-term exposure to DeBDE has been found to induce thyroid hyperplasia, hepatocellular and thyroid adenomas, and carcinomas in mice (Great Lakes Chemical Corporation, undated; 1987).

Reproductive and developmental effects
The estrogenic potency of several BDEs was tested using the estrogen receptor (ER)-CALUX bioassay, and the most potent congeners in descending order were BDEs 100, 75, 51, 30, and 119 (Brouwer *et al*., 2001; Meerts *et al*., 2001). Potency was much less than for estrogen. BDEs 166 and 190 were antiestrogenic.

Neonatal exposure to 2,2',4,4'-TeBDE (BDE47) (10.5 µg/g body weight), 2,2',4,4',5-PeBDE (BDE99) (0.8 or 12 µg/g body weight) or 2,2',4,4',5,5'-HxBDE (BDE153) (0.9 or 9 µg/g body weight), administered orally to mice on day 10, induced permanent aberrations in spontaneous motor behavior which worsened with age (Eriksson *et al.,* 2001; Viberg *et al*., 2001a; 2002). Neonatal exposure to BDE99 (12 µg/g body weight) or BDE153 (0.9 or 9 µg/g body weight) also affected learning and memory functions in the adult animal. BDE209 (2.22 or 20.1 µg/g body weight) administered orally to neonatal mice on day 3 induced permanent aberrations in spontaneous motor behavior, but when administered on day 10, had no effect (Viberg *et al*., 2001b). BDE99 exposure (0.6, 6 and 30 µg/g body weight/day) during pregnancy to post-natal day 21 in mice caused increased hyperactivity in offspring (Branchi *et al*., 2002).

In a follow-up study to Eriksson *et al.* (2001), Eriksson *et al.* (2002) investigated whether there is a critical time in neonatal mouse brain development for induction of the neurotoxic effects of BDE99. One single oral dose of 8 µg/g body weight (14 µmol/kg body weight) was administered to 3-day, 10-day and 19-day-old mice. The mice exposed to BDE99 on day 10 showed significant behavior aberrations, as was previously seen in Eriksson *et al.* (2001), and mice exposed on day 3 showed similar aberrations but to a lesser degree. The mice exposed on day 19 showed no significant change from the controls.

Uptake and retention of BDE99 in the brain was also studied by administering ^{14}C-labelled BDE99 to 3-day, 10-day and 19-day-old mice (Eriksson *et al*., 2002). The retention of BDE99 in mice exposed on day 3 indicates that the effects observed may be due to the amount of BDE99 still present in the brain on day 10. The neurotoxic effects seem to involve changes in the cholinergic system as mice given BDE99 on day 10 and then challenged as adults with a low dose of nicotine behaved

completely the opposite of controls. From these studies, it was concluded that the window for permanent effects of BDE99 and BDE47 is day 10 in neonatal mice (Eriksson *et al.*, 2001; 2002).

In Sinjari *et al.* (1998b), female rats given BDE47 orally for a period of two weeks were then killed and the choroid plexus of the brain removed, homogenized and incubated with ^{125}I-T4. Compared to controls, there was a dose-dependent reduction in the binding of ^{125}I-T4 to the choroid plexus. In contrast, *in vitro* incubation of rat choroid plexus with BDE47 revealed no competitive inhibition of labeled T4 binding. This indicates that BDE47 metabolites can cross the blood–brain barrier and bind to the choroid plexus T4-binding sites. This in turn could cause interference of T4 transport to the brain, with risks for effects on neural development.

BDE99 at 10 µM induced death of 23% of cerebellar neurons in an *in vitro* neurotoxicity test (Llansola *et al.*, 2001). Higher concentrations induced more neuronal death.

Microinjection of BDE47, 2,2',3,4,4'-PeBDE (BDE 85) or BDE99 into newly fertilized rainbow trout eggs in an early life stage mortality bioassay showed no effects compared to TCDD (Hornung *et al.*, 1996). Exposure to low levels of BDE47 affected developmental rates in the invertebrate *Acartia tonsa*, and juveniles were more sensitive than adults (Breitholtz *et al.*, 2001). This may implicate disruption of juvenile hormones or ecdysteroids.

Cytochrome P450-dependent monooxygenases

Using a recombinant H-4-II E rat hepatoma cell line having Ah receptor mediated expression of a luciferase reporter gene (the dioxin receptor (DR)-CALUX assay) (Aarts *et al.*, 1995; Murk *et al.*, 1998), a number of individual PBDE congeners have been tested for their potency to activate/deactivate the Ah receptor (Meerts *et al.*, 1998). In order to study antagonism, the same PBDE congeners were also tested in the presence of TCDD. Of the 17 PBDE congeners tested, seven (BDEs 32, 85, 99, 119, 153, 166, 190) showed ability to activate the Ah receptor. Potencies could only be determined for BDEs 166 and BDE190 and are in the same range as mono-*ortho* PCB congeners 105 and 118 (Sanderson *et al.*, 1996). Some congeners such as BDEs 85, 99, and 119 showed both agonist and antagonist activities depending on the concentration tested. Nine congeners, including BDEs 15, 28, 47, 77, and 138, showed antagonist activities against TCDD. The observed antagonism may be due to competition between PBDEs and TCDD at the Ah receptor level. In a recent study, more BDE congeners have been tested in the DR-CALUX assay and BDEs 30, 47, 51, 71, 75, and 100 have also been found to activate the Ah receptor, but their potencies could not be determined (Brouwer *et al.*, 2001).

EROD induction was studied in chick and rat hepatocytes, liver cell lines from rainbow trout, rats and humans, and in a human intestinal cell line (Chen *et al.*, 2001). BDEs 77, 100, 119 and 126 induced the greatest EROD activity in all cell types, but were less potent than TCDD. BDEs 153 and 183 were weaker inducers. BDEs 47 and 99 were not inducers in any cell line. The congeners that did not induce EROD also failed to bind to the Ah receptor.

Studies in whole cultured chick embryo liver showed induction of EROD activity after exposure to BDEs 47, 99, and 153 as well as the commercial mixture Bromkal 70-5 DE (Pettersson *et al.*, 2001). BDE99 was most potent but was much less potent than TCDD (TEF of 0.000004).

Microsomal enzyme activities were studied in rats and mice, and results from Bromkal 70-5 DE, and BDE 47 showed induction of EROD, MROD, and PROD in both species (Hallgren and Darnerud, 2002; Hallgren *et al.*, 2001). Rats exposed to Bromkal 70-5 DE orally for 28 days had dose-dependent increases in EROD and PROD activities (Fattore *et al.*, 2001).

Rainbow trout dosed orally with BDE47 or BD99 for 6 and 22 days had significantly inhibited EROD activity in the liver with BDE47 being most powerful in this effect (Tjärnlund *et al.*, 1998).

Immunosuppression

Mitogen-induced DNA synthesis and immunoglobulin synthesis by human lymphocytes *in vitro* were examined after exposure to purified BDEs 47 and 85. No effects on mitogen-induced proliferation or immunoglobulin synthesis were observed (Fernlöf *et al.*, 1997). The results indicate that proliferation and immunoglobulin synthesis are insensitive to the direct action of PBDEs.

Immunotoxicity was studied after oral treatment with Bromkal 70-5 DE or BDE47 in rats and mice (Darnerud and Thuvander, 1998). In mice, BDE47 caused reduced splenocyte number as reflected in decreased numbers of CD45R+, CD4+, and CD8+ cells in spleens. In mice treated with Bromkal 70-5 DE, absolute numbers of double negative thymocytes were significantly lower than in controls, and mice also showed reduced production of IgG. No effects were seen in rats. Thus, BDE47 and Bromkal 70-5 DE, which contains BDE47, both seem to be immunotoxic in mice.

Thyroid and retinol effects

Seventeen PBDE congeners (BDEs 15, 28, 30, 32, 47, 51, 71, 75, 77, 85, 99, 100, 119, 138, 153, 166, and 190) were incubated individually with rat hepatic microsomes from rats treated with β-naphthaflavone, phenobarbital or clofibrate (Meerts *et al.,* 2000). The parent congeners and the metabolites formed were then tested for their ability to compete with T4 for binding to human TTR *in vitro*. Results showed no competition with the parent compounds, but considerable potency for several of the metabolites, indicating the metabolism of PBDE to hydroxylated PBDE. The results indicate that hydroxylated metabolites of PBDE may be potent competitors of T4 and could disrupt normal thyroid hormone function in wildlife and humans if present. No binding competition was seen for several of the higher brominated PBDEs such as BDEs 138, 153, 166 and 190 after incubation with the microsomes. This may indicate that these congeners are not readily metabolized.

The Bromkal 70-5DE product causes decreased thymus weight, increased liver/body weight ratios in mice and decreased T4 in rats and mice (Fowles *et al.*, 1994; Darnerud and Sinjari, 1996; Hallgren *et al.*, 2001). Decreases in T4 were also seen when rats and mice were treated with the single congener BDE47, but no effects on TSH were seen for BDE47 or Bromkal 70 (Darnerud

and Sinjari, 1996; Hallgren *et al*., 2001). Bromkal 70-5 DE and BDE47 also caused significant reductions in hepatic vitamin A concentrations in rats, and Bromkal 70-5 DE caused reductions of hepatic vitamin A concentrations in mice at high doses (Hallgren *et al*., 2001). Similarly, Bromkal 70-5 DE caused dose-dependent reduction in hepatic vitamin A in rats dosed orally for 28 days (Fattore *et al*., 2001).

In subsequent studies, the interactive effects of different organohalogen compounds (PCB, PBDE, and chlorinated paraffins (CP)) on T4 levels and microsomal enzyme activities were tested (Hallgren and Darnerud, 2002). Female rats were orally exposed to single compounds or combinations at isomolar concentrations daily over 14 days. The results show that PCBs (Aroclor 1254) and PBDEs (BDE47) significantly reduce the T4 levels in rats, in the actual exposure interval (6-18 mg/kg body weight/day), with Aroclor 1254 resulting in the strongest effect. EROD and MROD, but to a lesser extent PROD and UDPGT, activities were negatively correlated to T4 effects. Regarding the mixed BDE47 + CP group, a synergistic decrease in free T4 levels and increase in EROD activity was observed.

Cancer
BDE47 was shown to induce a statistically significant increase in intragenic recombination when studied in one of two tested *in vitro* assays using mammalian cells (Helleday *et al.,* 1999). This may indicate that BDE47 can induce cancer via a non-mutagenic mechanism.

3.3.1.6. **PFOS and PFOA**

PFOS is a surfactant with both lipophobic and hydrophobic properties. Therefore, it does not accumulate in lipids, but instead accumulates in the liver, gall bladder and the blood (where it binds to proteins). It is speculated that the body treats PFOS as a bile acid. The liver makes bile acids from cholesterol, which are excreted from the gall bladder into the intestine to facilitate the emulsification and uptake of fats in the gut. The bile acids are then recycled back into the liver via enterohepatic circulation. PFOS may also weaken cell membranes (Hu *et al.,* 2000). PFOA is used as a lubricant, detergent, and wetting agent (Guethner and Vietor, 1962).

Liver enlargement and reduced serum cholesterol levels are early responses to exposure to PFOS. Acute toxicity with 100% mortality was seen in rhesus monkeys fed 10 mg/kg/day for three weeks in one experiment and in rhesus monkeys fed 4.5 mg/kg/day for seven weeks in another (Seed, 2000). Even doses of 0.75 mg/kg/day led to changes in cynomolgus monkey (*Macaca fascicularis*) livers, reductions in blood cholesterol, disinterest in food, and death. PFOA also causes liver enlargement and increased liver lipid levels in rats and mice (Kawashima *et al*., 1995; Kudo and Kawashima, 1997; Kudo *et al*., 1999).

Reproductive and developmental effects
In a two-generation reproductive toxicity study of PFOS in rats, pup-survival in the first generation was significantly decreased in the two highest dose groups receiving 1.6 and 3.2 mg/kg/day (Seed, 2000). All first-generation pups in the high-dose group died within one day of birth, and close to one-third of first generation pups in the 1.6 mg/kg/day group died within four days of birth. Only pups in the 0, 0.1 and 0.4 mg/kg/day groups were carried to the second generation. For second-generation offspring, reductions in pup weight and reversible delays in reflex and physical development were seen in the high-dose groups. For the second-generation offspring, the NOAEL for reduced pup weight was determined to be 0.1 mg/kg/day and the LOAEL, 0.4 mg/kg/day. These doses corresponded to PFOS liver concentrations of 15 µg/g and 58 µg/g ww, respectively, in the rats.

In rabbits, PFOS caused maternal toxicity (decreased body weight gain) at a dose of 1.0 mg/kg/day or higher (Case *et al*., 2001). Levels causing maternal toxicity also led to increased abortions and reduced fetal weights.

Cancer
PFOA and PFOS are not mutagenic but are known to be liver tumor promoters in rats. Perfluorinated fatty acids such as PFOA and PFOS increase peroxisome levels and inhibit GJIC (Upham *et al.,* 1998). PFOS is almost as potent as PFOA in causing increased peroxisome proliferation (Sohlenius *et al.,* 1993). Several other perfluorinated compounds (perfluorooctanoic sulfonamide, perfluorohexane sulfonate) also affect GJIC (Hu *et al.,* 2001).

3.3.2. **Persistent organic pesticides**

3.3.2.1. **Update on toxaphene**

A review of the effects of technical toxaphene was given in the previous AMAP assessment report. The toxicity data available at that time were rather limited. The following is an update on relevant effects studies that have been published recently. For a recent review on toxaphene, including toxicology, see de Geus *et al.* (1999).

Reproductive and developmental effects
Technical toxaphene, T2 (Parlar 26), and T12 (Parlar 50) were tested for their estrogenicity using the MCF7-E3 human breast cancer cell model and were found to have weak estrogenic activity (Stelzer and Chan, 1999). T2 and T12 had lower proliferative effects on the cells than technical toxaphene, and T2 was more potent than T12. Effects of mixtures of the three indicated additive effects, and none of the compounds had effects on estrogen receptor or progesterone receptor levels. In another study using MCF-7 cells, technical toxaphene, T2, and T12 were tested for their effects on estrogen receptor function (Jørgensen *et al.,* 1997c). The results indicated that toxaphene and T12 are antiestrogens, and that the effects occur at the gene transcription level. In support of this, toxaphene tested in a battery of assays for estrogenic activity (MCF-7 cells, competitive receptor binding) was found to be weakly antiestrogenic (Arcaro *et al.,* 2000). In a battery of estrogenic screening methods, including mouse uterus, MCF-7 human breast cancer cells, and yeast-based reporter gene assays, toxaphene was found to exert minimal estrogenic effects (Ramamoorthy *et al.,* 1997).

Technical toxaphene and congeners T2 and T12 were tested for their effects on cultured rat embryos during the period corresponding to a critical period of morphogenesis and organogenesis (gestational days 10-12).

Technical toxaphene and both single congeners caused significant changes in total morphology, somite number, head and crown-rump length, and central nervous system scores of the embryos, including a high incidence of central nervous system defects (Calciu *et al.,* 1997). The altered total morphology, including decreases in head and crown-rump lengths indicate that toxaphene and the two single congeners retard growth and morphological development. There were differences in potency and type of toxicity, indicating that specific congeners can produce effects not predicted by the technical mixture.

Technical toxaphene fed to female zebrafish for two weeks at doses of 20, 230, and 2200 ng/g body weight/day was found to have no effects on total number of eggs spawned, percentage of fertilized eggs, percentage of embryo mortality or percentage of hatching (Fåhraeus-Van Ree and Payne, 1997). Toxaphene, however, did cause a dose-related decrease in the percentage of oviposition.

The ability of toxaphene to displace native ligands from the estradiol receptor, testosterone receptor and cortisol receptor was tested using rainbow trout liver and brain tissues (Knudsen and Pottinger, 1999). Toxaphene did not bind to any of the receptors and was concluded not to be estrogenic.

In red-eared slider turtle, incubation temperature determines the sex of hatchlings, but male-producing temperatures can be overridden if the eggs are exposed to estrogenic compounds. Toxaphene was tested for its estrogenic activity by application on eggs set to become males (Willingham and Crews, 1999). No estrogenic effects were seen for toxaphene. Similarly, toxaphene showed no estrogenic effects when tested for its ability to displace native estradiol from alligator or human estrogen receptors (Vonier *et al.,* 1996; Arnold *et al.,* 1997). However, Palmer *et al*. (1998) found that water exposure to toxaphene induced significant vitellogenin production in the male African clawed frog (*Xenopus laevia*), which is an estrogenic effect.

Cytochrome P450-dependent monooxygenases

Subacute levels of toxaphene given to guinea pigs led to induced cytochrome P450 and increased aniline hydroxylase in the liver and kidney (Chandra and Durairaj, 1993). In mice, toxaphene exposure led to increases in CYP2B levels (Hedli *et al.,* 1998). Rats and Japanese quail exposed to single doses of technical toxaphene ranging from 0.012 to 40 mg/kg body weight showed induced P450 systems only at the highest dose (Drenth *et al.,* 2000). These included increased PROD, formation of 15β-hydroxytestosterone and 2-hydroxyestradiol in the rat, and increased formation of 6β-, 15α- and 16β-hydroxytestosterone in the quail. The doses required to induce the P450 system were close to those known to cause mortality, so it was concluded that P450 activity induction is probably unlikely in wildlife exposed to toxaphene.

In cynomolgous monkeys, toxaphene treatment induced aminopyrene, MROD, and EROD activities, indicating that toxaphene is a mixed-type inducer that induces both CYP1A and 2B (Bryce *et al*., 2001). Liver enzyme induction occurred at doses that did not cause any toxic effects in the monkeys, indicating that they may be less sensitive to the toxic effects of toxaphene than laboratory rodents.

Immunosuppression

Cynomolgus monkeys were treated with toxaphene at 1 mg/kg body weight/day for 52 weeks to study immune effects (Tryphonas *et al.,* 2000). Effects were seen that were not statistically significant, but which indicated possible negative effects of long-term exposure to toxaphene on humoral immunity.

Thyroid and retinol effects

Toxaphene caused thyroid follicular cell hypertrophy, intrafollicular hyperplasia and increased production of TSH in rats given 100 mg/kg/day for three days (Waritz *et al.,* 1996). No changes were seen for T3 or T4. The mechanism proposed for this effect was that toxaphene induced cytochrome P450 enzymes (CYP2B type) which led to increased excretion of T4, thereby stimulating the pituitary gland to produce and excrete more TSH. This, in turn, led to thyroid hypertrophy and hyperplasia.

Cancer

Toxaphene is a potent carcinogen in rats and mice (reviewed in Reuber, 1979; Saleh, 1991; de Geus *et al*., 1999). Toxaphene induces malignant liver tumors, reticulum cell sarcomas, uterine sarcomas, reproductive system tumors, mammary gland tumors, and tumors in the pituitary, adrenal and thyroid glands.

Toxaphene is mutagenic in the Ames test (Hooper *et al.,* 1979; Mortelmans *et al.,* 1986). When toxaphene and four single congeners (Parlars 26, 50, 62, and 32) were tested for mutagenicity using two different bacterial (*Salmonella typhimurium*) strains, toxaphene was mutagenic but the single congeners were not (Steinberg *et al.,* 1998). Schrader *et al.* (1998) found that technical toxaphene was mutagenic in all five *S. typhimurium* strains tested but high concentrations were required. However, no mutagenesis was seen for toxaphene when tested in Chinese hamster V79 lung fibroblasts. Toxaphene and Parlar 32 were found to be genotoxic using the Mutatox assay, but Parlars 26, 50, and 62 were not genotoxic (Boon *et al.,* 1998).

Low concentrations of toxaphene induce micronuclei formation *in vitro* in beluga skin fibroblasts (Gauthier *et al*., 1999).

Non-cytotoxic concentrations of toxaphene inhibited GJIC in normal human breast epithelial cells, in a dose-dependent manner (Kang *et al.,* 1996), indicating it may be a tumor promoter. No DNA adducts were found in the livers of mice treated with toxaphene (Hedli *et al.,* 1998). Toxaphene has been found to down-regulate the retinoblastoma gene, a tumor suppressor gene, indicating that toxaphene could promote tumors by turning off tumor suppression (Rought *et al.,* 1999).

3.3.3. Other pesticides

3.3.3.1. TBT and its metabolites (DBT, MBT)

TBT is one of the most toxic substances deliberately introduced into natural waters (Goldberg, 1986). TBT is moderately lipophilic and bioconcentrates and bioaccumulates in the marine environment (Tanabe, 1999; Maguire, 2000; Hoch, 2001), and is a classic endocrine disrupter (Matthiessen and Gibbs, 1998). For recent reviews on TBT toxicology, see Fent (1996a), Maguire (2000) and WHO (1999). Chronic effects are observed

at exposure levels of 1000 ng/L or less for oysters, mussels and crustaceans (Rexrode, 1987), while the most sensitive species (dogwhelk snails, e.g., *Nucella lapis*) show sublethal effects at concentrations of only a few ng/L or less (Bryan and Gibbs, 1991; Gibbs, 1993; Stewart and Thompson, 1994).

Recently (since the mid-1990s), studies have been published showing the occurrence of TBT and other butyltins (MBT, DBT) in fish, birds, and terrestrial and marine mammals from the Pacific Ocean (Japan, Australia, Taiwan, India, Bangladesh, Thailand, Vietnam, Indonesia, Alaska, U.S., and open ocean areas) (Iwata *et al*., 1995; Kannan *et al*., 1995a; 1995b; Guruge *et al*., 1996;1997; Kim *et al*., 1996a; 1996b; 1996c; Takahashi *et al*., 1997; 1999; Tanabe *et al*., 1998), in the Baltic Sea (Kannan and Falandysz, 1997), on the U.S. Atlantic and Gulf coasts (Kannan *et al*., 1997), in a freshwater lake in the Netherlands (Stäb *et al*., 1996), and along the coast of Italy (Kannan *et al*., 1996).

Butyltins concentrate in the liver, blubber, and muscle of vertebrates. Higher relative amounts of MBT and DBT are found in birds and mammals as compared to fish, due to metabolism of TBT.

Reproductive and developmental effects

TBT has been found to have high binding affinity to the androgen receptor in an *in vitro* assay but shows no affinity for the estrogen receptor (Satoh *et al*., 2001).

At concentrations of 1-2 ng/L, dogwhelk snails exhibit imposex. Imposex is caused by TBT interference with the biosynthesis of steroid hormones (i.e., the synthesis of 17β-estradiol from testosterone and the synthesis of estrone from androstenedione) (Bettin *et al*., 1996). High levels of testosterone result in the development of a penis and vas deferens in female neogastropods. At levels of 7-10 ng/L, the vas deferens can overgrow the genital opening of the female, resulting in reproductive failure of the species (Gibbs *et al*., 1987). Thresholds for other species are poorly known. Studies with cod (*Gadus morhua*) (Granmo *et al*., 2002) indicate that threshold levels for cod embryos are higher than found in the Arctic environment.

Imposex has been observed most frequently in coastal areas near obvious TBT sources such as marinas or harbors, and has been associated with TBT paints on both pleasure boats and commercial shipping. Sediments in particular seem to be reservoirs for TBT especially after use has stopped, leading to continued exposure (Maguire, 2000). Open ocean areas are exposed to TBT from large vessels that are still allowed to use TBT on their hulls, with imposex being found in whelks (*Buccinum undatum*) collected from the open North Sea along shipping routes, indicating that the problem is not confined to coastal areas (Ellis and Pattisina, 1990; Bryan and Gibbs, 1991; Ten Hallers-Tjabbes *et al.,* 1994).

TBT causes developmental effects in the early life stages of fish. Water concentrations of 690-820 ng/L cause scoliosis and an inability to swim in minnows (*Phoxinus phoxinus*) (Fent, 1992) and notochord length is significantly reduced in larval striped bass (*Morone saxatilis*) at 514 ng/L (Pinkney *et al.,* 1990). Long-term exposure of rainbow trout yolk sac fry to 1000 ng/L resulted in decreased numbers of red blood cells, increased liver weight, and liver cell hyperplasia (Seinen *et al.,* 1981). A NOAEL of 40-50 ng/L for rainbow trout yolk sac fry has been proposed (De Vries *et al.,* 1991). TBT exposure in medaka embryos on day 0 led to concentration-related lethality, reductions in hatching success, increases in gross abnormalities (bent, curled and/or shortened tails caused by reduced number of somites) and slowed developmental rates (Bentivegna and Piatkowski, 1998). The LOAEL for TBT combined chronic effects in medaka embryos was 12 500 ng/L.

TBT-oxide (TBTO) exposure (0, 2.7 and 9 ng/ml) for four days led to significant changes in spatial position, response to predator attack, recovery time and latency time in three-spined stickleback (Wibe *et al*., 2001).

Dietary exposure of Japanese quail (*Coturnix coturnix japonica*) to 60 000 and 150 000 ng TBTO/g food led to reduced egg hatchability and an increase in the percent of chicks found dead in the shell (Coenen *et al.,* 1992; Schlatterer *et al.,* 1993). The no-observed-effect concentration (NOEC) for reduced egg weight and hatchability was 60 000 ng/g food (Schlatterer *et al.,* 1993).

Cytochrome P450-dependent monooxygenases

TBT exposure to 3.3, 8.1 and 16.3 mg/kg (intraperitoneal injection) led to the concentration-dependent degradation of CYP1A and loss of EROD activity *in vitro* and *in vivo* in scup (*Stenotomus chrysops*) (Fent and Stegeman, 1991; 1993; Fent *et al.,* 1998). The lowest dose level resulted in liver TBT concentrations of 8000 ng/g. At the high dose levels used, CYP2B (responsible for testosterone 15α-hydroxylase activity) and CYP3A (responsible for testosterone 6β-hydroxylation) proteins were also destroyed, which could lead to effects on steroid metabolism and possible endocrine disruption.

TBT exposure *in vitro* in rainbow trout, bullhead (*Cottos gobio*), and eel (*Anguilla anguilla*) hepatic microsomes led to strong inhibition of EROD activity and reduction in total cytochrome P450 protein levels (Fent and Bucheli, 1994), with rainbow trout microsomes being most sensitive. Rainbow trout hepatocytes also showed reduced cytochrome P450 levels after exposure to 1 µM TBT (290 000 ng/L) (Reader *et al.,* 1996). In the fish hepatoma cell line PLHC-1, TBT exposure led to inhibition of EROD, and decreased levels of CYP1A and DBT exposure also led to inhibition of EROD, but was less potent than TBT (Brüschweiler *et al.,* 1996). These studies indicate that TBT is metabolized by the P450 monooxygenase system but that it also inhibits this system, affecting its own metabolism and that of other substances. TBT and DBT both inhibit carboxylesterases in the tropical marine fish *Siganus canaliculatus*, with DBT being most potent (Al-Ghais *et al.,* 2000). The IC_{50}s (concentrations at which 50% inhibition occurs) were 180-385 µM (52 200-112 000 ng/g) for TBT and 17-49 µM (4000-11 400 ng/g) for DBT.

In vitro inhibition of hepatic cytochrome P450 in Dall's porpoise (*Phocoenoides dalli*) and Steller sea lion hepatic microsomes by TBT has also been shown (Kim *et al.,* 1998b). Total P450 levels decreased and EROD and PROD activity decreased with increasing doses. The apparent effect threshold concentration was estimated to be 100 µM TBT (29 000 ng/g). Comparison of the composition of TBT and its metabolites DBT and MBT

in marine mammals and their prey indicates that pinnipeds (seals, sea lions) have a higher metabolic capacity for organotins than cetaceans (dolphins, porpoises, whales) (Tanabe *et al*., 1998; Tanabe, 1999). Both TBT and DBT are hepatotoxic in mice, with DBT being more potent than TBT (Ueno *et al.,* 1994). TBT toxicity in this study was attributed to the metabolism of TBT to DBT and subsequent accumulation of DBT. The effect threshold for hepatotoxic effects in mice was 2600 ng DBT/g ww in liver.

Immunosuppression

Both TBT and DBT have been found to be immunosuppressive in a range of animals, causing thymic and splenic atrophy, reductions in thymic, circulating and splenic lymphocytes, suppression of T-cell-dependent immunity, and suppression of tumoricidal activity (Seinen *et al.,* 1977; Krajnc *et al.,* 1984; Vos *et al.,* 1984; Snoeij *et al.,* 1985; 1988). For a review of organotin immunotoxicity, see Snoeij *et al.* (1987). DBT and TBT also suppressed concanavalin-A-induced mitogenesis in peripheral blood monocytes of marine mammals (Nakata *et al*., 2002). *In vitro* immunotoxicity is seen in rat thymocytes (Snoeij *et al.,* 1986) and rabbit polymorphonuclear leucocytes (Elferink *et al.,* 1986) with an effect threshold less than 1.0 µM TBT (290 ng/g ww) in both studies. A NOEL of 500 ng/g body weight has been proposed for TBTO immunotoxicity in rats (Verdier *et al*., 1991). The most sensitive *in vivo* effect of TBT-induced immunosuppression is seen in weanling rats, as measured by reduced IgE titers and reduced resistance to parasitism by *Trichinella spiralis* in muscle (Vos *et al.,* 1990). The NOAEL was determined to be 25 µg/kg/day (equivalent to 500 ng/g in diet), and the LOAEL was 250 µg/kg/day (equivalent to 5000 ng/g in diet).

One possible mechanism that has been proposed for TBT immunotoxicity is the induction of apoptosis, or programmed cell death, in the thymus (Pieters *et al.,* 1994). This may be related to alterations of Ca^{2+} homeostasis, formation of reactive oxygen species and DNA fragmentation (Gennari *et al.,* 2000).

TBT is also immunotoxic in rainbow trout immune cells, but DBT has been found to be more potent as an immunotoxin (O'Halloran *et al.,* 1998). Both TBT and DBT were found to suppress mitogenic activity in trout head kidney and splenic cells at exposure concentrations of 50 ng/g or greater (154 nM). Studies in rainbow trout *in vivo* also show similar types of effects to those seen in mammals, such as lymphoid depletion and immune modulation (Schwaiger *et al.,* 1992). In channel catfish (*Ictalurus punctatus*), TBT affected humoral immunity as measured by response to *Edwardsiella ictaluri* infection (Rice *et al.,* 1995). The effects were significant for all doses (10, 100, and 1000 ng/g administered intraperitoneally), but the strength of the response was dose-dependent. Flounder (*Platichthys flesus*) exposed to fairly high TBT concentrations (µg/L) had a significant decrease in thymus volume (Grinwis *et al.,* 1998).

In Canada, studies have shown effects of TBT, DBT, and MBT on the *in vitro* phagocytic activity of hemocytes from three marine bivalve species, *Mytilus edulis*, *Mya arenaria*, and *Mactromeris polynyma*, using flow cytometry (Bouchard *et al*., 1999). Phagocytosis was reduced with increasing doses of TBT and DBT, and the toxicity of butyltins on hemocytes decreased in the order DBT > TBT > MBT. The comparison of the relative sensitivity of the three species showed that blue mussels (*M. edulis*) were more tolerant of butyltin compounds than both clam species.

Cancer

Generally, negative results have been obtained when TBT is tested for mutagenicity in various test systems (WHO, 1990). However, Hamasaki *et al*. (1993) have shown that MBT, DBT and TBT were mutagens in *S. typhimurium* TA100. Carcinogenicity has not been well investigated. Since the data on genotoxicity of TBT are not consistent, it is not possible to draw definite conclusions.