# Canadian Environmental Quality Guidelines for Polychlorinated Dibenzo-*p*-dioxins and Polychlorinated Dibenzofurans

*Water, Sediment, and Tissue*

## Volume I: Guideline Derivation

# Draft Copy for Review Only

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## EXECUTIVE SUMMARY

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), commonly known as dioxins and furans, respectively, are planar tricyclic aromatic compounds. As a group, PCDD/Fs are considered toxic, persistence, and bioaccumulative. Environmental concentrations of PCDD/Fs are primarily the result of human activities. As these characteristics meet the specifications for a Track 1 substance under the Toxic Substances Management Policy, PCDD/Fs have been slated for virtual elimination from the Canadian environment. As mandated by the *Canadian Environmental Protection Act*, the Water Quality Guidelines Task Group of the Canadian Council of Ministers of the Environment (CCME) has developed herein Environmental Quality Guidelines for dioxins and furans for the protection aquatic and terrestrial wildlife. This report summarizes physical and chemical properties; sources and pathways; fate and persistence; environmental distribution and levels; and, toxicity to aquatic and terrestrial wildlife for the 17 PCDD/F congeners that have chlorine atoms attached at positions 2,3,7, and 8. Subsequently, ambient water quality guidelines (WQGs) and sediment quality guidelines (SQGs) for the protection of freshwater and marine/estuarine aquatic biota, and tissue residue guidelines (TRGs) for the protection of wildlife consumers of freshwater and marine/estuarine aquatic biota are derived using toxic equivalents (TEQs) according to formal, nationally approved protocols. These Environmental Quality Guidelines will form the scientific basis for ambient Canada Wide Standards for dioxins and furans developed under the Canada-Wide Environmental Standards Sub-Agreement of the *Canada-Wide Accord on Environmental Harmonization.*

PCDD/Fs are omnipresent in the air, soil, riverbeds, and biota, although they have never been intentionally produced, nor have any known use. They are involuntary impurities formed as a result of anthropogenic activities, including chemical manufacturing, waste incineration, petroleum refining, wood-burning, metallurgical processes, fuel combustion (automobiles), residential oil combustion, electric power generation, cement kilns, and biological incineration.

Natural sources include forest fires, volcanic activity, and other forms of natural combustion. One of the most significant sources of PCDD/Fs to the environment are municipal waste incinerators.

Owing to their hydrophobic nature, the majority of PCDD/Fs released into aquatic systems ultimately become associated with the organic fraction of suspended and/or bed sediments and lipid-rich tissues of aquatic organisms. PCDD/Fs which accumulate in sediments are chemically stable and therefore may persist for long periods of time. Thus, bed sediments may represent long term sources of PCDD/Fs to the aquatic food web as bioaccumulation from the organic fraction of the sediments is an important path of uptake of PCDD/Fs for some aquatic organisms (e.g., carp, *Cyprinus carpio*). Aquatic organisms may uptake PCDD/Fs from water, sediment, or through the consumption of contaminated prey items. All 2,3,7,8-substituted PCDD/Fs readily accumulate in the tissues of aquatic organisms, though higher chlorinated PCDD/Fs generally accumulate to a lesser degree than lower chlorinated congeners. Lipid-normalized bioconcentration factors ($BCFs_{lipid}$) recorded for $T_4CDD$ were the highest of all congeners, with a geometric mean of 175 245. The geometric mean biota-sediment accumulation factors (BSAFs) for $T_4CDD$ are 0.3 and 0.14 for freshwater and marine/estuarine systems, respectively. Accumulation from food may be the primary source of PCDD/Fs for some species (e.g., lake trout, *Salveinus namaycush*) but not for others (e.g., carp and guppies, *Poecilia reticulata*). Moreover, PCDD/Fs seem unusual in that they do not appear to biomagnify like other halogenated aromatics with comparable hydrophobicities. The greatest biomagnification factors (BMFs) reported were 32 and 76 for herring gulls (*Larus argentatus*) and mink (*Mustela vison*), respectively. More research on accumulation rates and processes is needed to obtain a clearer understanding of PCDD/F movement through the food chain.

PCDD/Fs are thought to elicit most, if not all, of their toxicity via the aryl hydrocarbon (*Ah*) receptor, a protein conserved across mammals, birds, and fish. A multitude of toxic and biologic responses to PCDD/F exposure have been described in the scientific literature and include:

mortality (often delayed), decreased body weight gain, decreased feed consumption, thymic atrophy, histopathologic effects, immunotoxicity, developmental and reproductive effects, biochemical effects, neurotoxicity, and carcinogenesis. PCDD/Fs are also known to disrupt the endocrine system which could have serious repercussions on sexual development. Clearly, it is unlikely that the complete spectrum of effects would be observed in any single species but the data indicate that PCDD/Fs and related compounds elicit the same qualitative pattern of responses within each species. For the purposes of developing Canadian Environmental Quality Guidelines, toxic responses of aquatic biota (plants, invertebrates, amphibians, and fish), and wildlife (mammals and birds) to PCDD/Fs exposure through water, sediment, and/or diet were reviewed and evaluated.

In order to compare toxicities of environmental samples or experimental test doses that have different congener profiles, toxic equivalency factors (TEFs) have been developed. *Ah* receptor compounds are assigned TEFs based on their ability to induce a response in the cytochrome enzyme system relative to the most potent inducer, $T_4CDD$. Within a sample, individual chemical concentrations are multiplied by their respective TEFs and all products are summed to give a value expressed in toxic equivalency units (TEQs). This method takes into consideration the unique concentrations and toxicities of the individual components within a chemical mixture but nevertheless is unable to account for non-additive interactions among different chemicals that are known to occur. The most recent TEF values for mammalian, avian, and fish species developed by the World Health Organization (van den Berg et al. 1999) were applied in this document.

The Canadian WQGs were developed in accordance with the formal protocol (CCME 1991a). A WQG of 0.038 pg TEQ·$L^{-1}$ for total PCDD/Fs is recommended for the protection of freshwater life. This value was calculated by applying an uncertainty factor of 0.001 to a LOEL of 0.038 ng TEQ·$L^{-1}$ for growth (after 28 days exposure) and for mortality (for 28 days exposure and 28 days depuration) in juvenile rainbow trout, *Oncorhynchus mykiss* (Mehrle et al. 1988).

Sufficient supporting information is available to endorse a full WQG for freshwater. As no data were located on the toxicity of PCDD/Fs to marine or estuarine organisms, the WQG for freshwater of 0.038 pg TEQ·$L^{-1}$ for total PCDD/Fs is adopted as the interim WQG for the protection of marine and estuarine life.

At present, full Canadian SQGs for PCDD/Fs can not be recommended according to the formal protocol (CCME 1995). Sufficient toxicological data for freshwater sediments exist, however, to derive a threshold effect level (TEL) of 10 ng TEQ·$kg^{-1}$ dw and a probable effect level (PEL) of 189 ng TEQ·$kg^{-1}$ dw using the modified National Status and Trends Program (NSTP) approach. An evaluation of the incidence of effects observed below the freshwater TEL and above the freshwater PEL indicates that the narrative objectives for these values have been met according to the formal protocol. According to formal protocol, the TEL is recommended as the interim freshwater SQG and the PEL as an additional assessment tool. A detailed assessment, however, of the guideline derivation tables indicates that the data used to calculate the TEL and PEL may not adequately represent a diverse body of evidence regarding effects of sediment associated PCDD/Fs. To address this issue, two equilibrium approaches were informally evaluated. Sediment quality assessment values (SQAVs) developed using the water-sediment equilibrium partitioning (EqPA) approach and the tissue residue-based equilibrium partitioning (TRB-EqPA) approach were at least an order of magnitude less than the TEL, indicating that the TEL may not adequately protect aquatic organisms. Therefore, an uncertainty factor of 0.1 was applied to the TEL; this adjusted TEL of 1 ng·$kg^{-1}$ dw is recommended as the interim freshwater SQG, and is believed to be a better estimate of the concentrations of sediment-associated PCDD/Fs that will not harm aquatic organisms associated with bed sediments over an indefinite period of exposure. The PEL was similarly adjusted to 18.9 ng·$kg^{-1}$ dw and is recommended as an additional assessment tool. Both the interim SQG and the adjusted PEL apply to surficial sediments (i.e., top 0 to 5 cm).

Because there was insufficient data available to support the derivation of a marine SQG, the

interim freshwater SQG value of 1 ng TEQ·kg$^{-1}$ dw and the adjusted PEL of 18.9 ng·kg$^{-1}$ dw are provisionally recommended as the interim marine SQG and the adjusted marine PEL for surficial sediments (i.e., top 0 to 5 cm), respectively.

For the derivation of the Canadian TRG, the lowest tolerable daily intake (TDI) levels for PCDD/Fs were divided by the highest food intake rate to body weight ratios (FI:BW) for both mammalian and avian species to derive reference concentrations (RCs). The lowest RC among available mammalian and avian species is that for female mink (*Mustela vison*), 0.71 ng TEQ·kg$^{-1}$ diet; this value is adopted as the TRG for PCDD/Fs. The guideline refers to the TEQ concentration due to PCDD/Fs measured in an aquatic organism on a wet weight basis that is not expected to result in adverse effects on wildlife. This guideline is considered interim as avian toxicity data was only sufficient to satisfy minimum requirements for an interim guideline. Because no dietary toxicity data were located for amphibian and reptilian species, this interim guideline applies only to mammalian and avian wildlife.

**Summary of Canadian Environmental Quality Guidelines for Dioxins and Furans**

| Media | Guideline* |
|---|---|
| Aquatic Life | |
| *freshwater* | 0.038 pg TEQ·L$^{-1}$ (full) |
| *marine* | 0.038 pg TEQ·L$^{-1}$ (interim) |
| Sediment Quality | |
| *freshwater* | 1 ng TEQ·kg$^{-1}$ dw (interim) |
| *marine* | 1 ng TEQ·kg$^{-1}$ dw (provisional interim) |
| Tissue Residue | 0.71 ng TEQ·kg$^{-1}$ diet, ww (interim) |

*values are expressed as toxic equivalency (TEQ) units, based on TEF values from the World Health Organization (van den Berg et al. 1998).

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## GLOSSARY

**absorption** (1) The taking up of chemical substances by organisms through ingestion, dermal contact, or gaseous exchanges. (2) The process by which one material takes up and retains another through the penetration of the absorbed molecules into the mass of the absorbing material.

**acute** (1) Having a sudden onset, lasting a short time. (2) Of a stimulus, severe enough to induce a response rapidly. Can be used to define either the exposure or the response to an exposure (effect). (3) A brief exposure to a stressor or the effects associated with such an exposure. It can refer to an instantaneous exposure (i.e., oral gavage) or continuous exposures, from minutes to a few days depending on the life span of the organism.

**acute toxicity** A toxic effect (severe biological harm or death) produced in an organism by a substance or mixture of substances within a short exposure period (usually 96 hours or less).

**additive toxicity** The toxicity of a mixture of chemicals that is approximately equivalent to that expected from a simple summation of the known toxicities of the individual chemicals present in the mixture (i.e., an algebraic summation of effects).

**adsorption** The process by which one material takes up and retains another through the bonding of the adsorbed molecules onto the surface of the adsorbing material.

**ambient** The conditions in the surrounding environment.

**ambient concentration** Representative level of a contaminant in an area. May reflect natural geologic variations or the influence of generalized industrial or urban activity in a region, presumably unaffected by point sources of contaminant release.

**antagonism** A phenomenon in which the toxicity of a mixture of chemicals is less than that which would be expected from a simple summation of the toxicities of the individual chemicals present in the mixture (i.e., an algebraic subtraction of effects).

**anthropogenic** Refers to the activities of humans or the alterations resulting from them.

**assimilation** (1) In cells, the incorporation of absorbed substances (e.g., foodstuff) for growth

and reproduction. (2) The capacity of a mass of air or body of water to dilute the release of pollutants.

**benchmark concentration** Specific concentrations at which some level of effects is expected (e.g., $LC_{25}$ and maximum acceptable toxicant concentration). These concentrations are derived from hazard assessment.

**benthic** Refers to the substrate at the bottom of aquatic habitats (e.g., lakes, oceans, and rivers). Also describes the life strategy of organisms living in or on that substrate (e.g., clams and oligochaete worms).

**bioaccumulation** The process by which chemical substances are accumulated by organisms from exposure to water, sediments, or soil directly or through consumption of food containing the chemicals.

**bioaccumulation factor (BAF)** The ratio of the concentration of a given compound in the tissues of an organism and its concentration either in the media in which the organism lives or in the tissues of biota on which the organism feeds.

**bioassay** Test used to evaluate the relative potency of a chemical by comparing its effect on living organisms or parts thereof (e.g., cell culture) with the effect of a control.

**bioavailable** The fraction of the total chemical in the surrounding environment that can be taken up by organisms. The chemical may be dissolved or reversibly bound to particles in water, air, sediment, or soil, or contained in food items.

**bioconcentration** The process by which contaminants are directly taken up by organisms from the medium in which they live.

**bioconcentration factor (BCF)** The ratio of the concentration of a given compound in the tissues of an organism and its concentration in the media in which the organism lives (e.g., water). The ratio reflects the apparent equilibrium stage of the uptake phase during a bioconcentration test.

**biodegradation** The aerobic or anaerobic breakdown of organic substances, including organic contaminants, by biota such as microbes and fungi.

**biological monitoring** The direct measurement of changes in the biological component of a

habitat, based on physiological, behavioural, reproductive, or other responses in organisms, relative to environmental changes in time or space. Other common responses measured include contaminant levels in tissue and changes in the taxonomic composition of assemblages.

**biomagnification** The increase in tissue concentrations of accumulated chemicals from one trophic level to the next (i.e., organisms contain higher concentrations of the substance than their food sources).

**biomagnification factor (BMF)** A ratio of BAFs and reflect the extent to which BAFs increase with trophic level.

**biota** Biological organisms (e.g., plants, microbes, fish, and wildlife).

**bioturbation** The physical disturbance of sediments by burrowing and other activities of organisms.

**carcinogen** A substance that can potentially induce cancer in a living organism.

**chlorination** (1) The process of introducing one or more chlorine atoms into a compound. (2) The application of chlorine to water, sewage, or industrial wastes for disinfection or for other biological or chemical results.

**chronic** Involving a stimulus that is lingering or continuous over a long period of time; often signifies periods varying from several weeks to years, depending on the reproductive life cycle of the species. Can be used to define either the exposure or the response to an exposure (effect). Chronic exposure typically induces a biological response of relatively slow progress and long continuance.

**chronic toxicity** A toxic effect produced in an organism by a substance or mixture of substances over a long exposure period.

**clay** Soil and sediment particles of equivalent diameter <0.002 mm usually consisting of clay minerals but commonly including amorphous free iron oxides and primary minerals.

**clay mineral** Finely crystalline hydrous aluminum silicates and hydrous magnesium silicates with a phyllosilicate structure.

**community** An assemblage of organisms characterized by a distinctive combination of species occupying a common environment and interacting with one another.

**congener** A compound belonging to a family of compounds having similar chemical skeletons but differing in the number and position of hydrogen substitutes (e.g., PCDDs and PCDFs).

**contaminant** Any chemical substance whose concentration exceeds background concentrations or that does not naturally occur in the environment

**control** A treatment in a toxicity test or in a field study that duplicates all the conditions of the exposure treatments or test sites except that the control contains no test substance. This determines the absence of toxicity under the basic test conditions. It is often called negative control treatment to differentiate it from positive control treatments. In a positive control treatment, a chemical known to elicit the desired response is added to the exposure medium of this treatment to demonstrate the validity of the measured endpoint under the tested conditions and the absence of toxicity in presence of the test substance.

**criteria** Numerical value(s) or narrative statement for a physical, chemical, or biological characteristic of water, biota, soil, or sediment that must not be exceeded to protect, maintain, and improve the specific uses of soil, sediment, and water.

**delivered dose** The amount or concentration of a substance at the targeted site within the body. The delivered dose may consider metabolic activation processes, pharmacodynamics, and tissue dosimetry.

**detection limit** The smallest concentration or amount of a substance that can be reported as present in a sample with a specified degree of certainty by a definite, complete analytical procedure.

**detritus** Unconsolidated sediments composed of inorganic and decaying organic material.

**dissolved constituent** The constituents of a water sample or a soil, sediment, or tissue digestate that will pass through a 0.45-µm membrane filter.

**dose** The quantifiable amount of a material introduced into an animal.

**early life-stage test (ELS)** Toxicity test on the early life stages of a species, from shortly after fertilization through embryonic, larval, or early juvenile development. Data are obtained on survival and growth.

**ecosystem** An ecological system. A natural unit of living and nonliving components that interact

to form a stable system in which a cyclic interchange of material takes place between living and nonliving units.

**effective concentration [median~] ($EC_{50}$)** The concentration of a stressor that is estimated to be effective in producing a biological response, other than mortality, in 50% of the test organisms over a specific time interval (e.g., a 48-h daphnid $EC_{50}$).

**effective dose [median~] ($ED_{50}$)** The dose of material estimated to be effective in producing some sublethal response in 50% of the test organisms. It is appropriately used with test animals such as rats, mice, and dogs, but it is rarely applicable to aquatic organisms because it indicates the quantity of a material introduced directly into the body by injection or ingestion rather than the concentration of the material in water in which aquatic organisms are exposed during toxicity tests.

**endpoint measurement** An effect on an ecological component that can be measured and described quantitatively.

**excretion** Expulsion of metabolic wastes, sometimes including toxic substances, into the environment by microbes, animals, and plants.

**exposure** The amount of a physical or chemical agent that reaches a target or receptor through ingestion, dermal absorption, and inhalation.

**exposure assessment** The process of estimating the dose received by an organism, population, or ecosystem. It may be prospective where estimates of the chemical concentrations and forms in various media or habitats are combined with estimates of the organism's behaviour to predict dose, or it may also be retrospective where dose is estimated from body burdens of the chemical or changes in the organism caused by the chemical (biomarkers).

**exposure characterization** Identification of the conditions of contact between a substance and an individual or population. Exposure characteristics may involve identifying the concentration, routes of uptake, target sources, environmental pathways, and population at risk.

**exposure estimation** Estimate of the amount and duration of contact between a substance and an individual or a population. Exposure estimates consider factors such as concentration, routes of uptake, target sources, environmental pathways, population at risk, and time scale.

**exposure route/pathway** The means by which organisms are exposed to contaminants. Routes/pathways would include uptake of contaminants from solution, ingestion of contaminated food or prey, and inhalation of contaminated particles. More generally, routes of exposure include exposure via air, water, soil, sediments, food, and other media to which the organism may be exposed.

**exposure scenario** A clearly and quantitatively defined description of all circumstances associated with a receptor that would permit the estimation of chemical exposure. These circumstances include amounts of air, food, water, and soil consumed, and the critical receptor's weight, age, sex, and all other relevant considerations.

**fate** The manner in which a material will partition between various environmental compartments (e.g., soil, sediment, water, air, or biota) as a result of transport, transformation, and degradation.

**flow-through system** An exposure system for aquatic toxicity tests in which control water and test material solution flow into and out of test chambers on a once-through basis either intermittently or continuously.

**guidelines** Numerical concentrations or narrative statements that are recommended to protect and maintain the specified uses of air, water, sediment, soil, or wildlife.

**hardness** The concentration of all metallic cations, except those of the alkali metals, present in water. In general, hardness is a measure of the concentration of calcium and magnesium ions in water and is frequently expressed as $mg \cdot L^{-1}$ calcium carbonate equivalent.

**humic substances** Partially broken down organic substances that occur in water or sediment, mainly in a colloidal state. Humic acids are large-molecule organic acids that dissolve in water.

**hydrolysis** (1) The formation of an acid and a base from a salt by the ionic dissociation of water. (2) The chemical decomposition of a compound by interaction with water.

**interim guideline** For sediment, water, and tissue residue guidelines: a guideline value derived from a small, less restrictive data set than that full for a guideline.

**in vitro** Outside the intact organism; generally applied to experiments involving biochemical events occurring in tissue fragments or fractions.

**in vivo** Within an intact animal or organism.

**$K_d$** The ratio of the concentration of a chemical associated with the particulate phase, the particulate phase being expressed as the total weight of particles, and the concentration of that chemical in the dissolved phase, at equilibrium.

**$K_{oc}$** Organic carbon (normalized) partition coefficient. The ratio of the concentration of a chemical associated with the particulate phase, the particulate phase being expressed as the weight of the organic carbon content, and the concentration of that chemical in the dissolved phase, at equilibrium. The logarithm of $K_{oc}$ is used as an indication of a chemical's propensity for accumulating in organic matter, such as humin or humic acid.

**$K_{ow}$** Octanol–water partition coefficient. The ratio of a chemical's concentration in *n*-octanol and its concentration in water at equilibrium. The logarithm of $K_{ow}$ is used as an indication of a chemical's propensity for bioconcentration in aquatic organisms.

**leaching** The process by which soluble constituents are gradually removed from soil through the action of percolating water.

**lethal concentration [median~] ($LC_{50}$)** The concentration of a stressor that is estimated to be lethal to 50% of the test organisms over a specific time interval (e.g., 96-h $LC_{50}$).

**lethal dose [median~] ($LD_{50}$)** The dose of material that is estimated to be lethal to 50% of the test organisms. It is appropriately used with test animals such as rats, mice and dogs, but it is rarely applicable to aquatic organisms because it indicates the quantity of a material introduced directly into the body by injection or ingestion rather than the concentration of the material in water in which aquatic organisms are exposed during toxicity tests.

**life-cycle study** A chronic study involving the entire reproductive cycle of an organism in which all the significant life stages of the organism are exposed to a test material. A partial life-cycle toxicity test includes that part of the life cycle that has been observed to be especially sensitive to chemical exposure.

**ligand** A non-metal ion, molecule, or atom that is attached to the central atom of a coordination compound, a chelate, or other complex, by donating one or more pair of electrons. May also be called complexing agent.

**long-term exposure** Exposure to a contaminant in a medium, lasting from several weeks to years, often encompassing the reproductive cycle or life cycle of the test organism. Usually referred to as a chronic exposure. Absolute definitions for this term vary among studies.

**lowest-observed-effect level (LOEL)** The lowest dose or concentration used in a toxicity test that results in statistically significant observed adverse effects in the exposed organisms compared with control organisms.

**measured flow-through test** A toxicity test with constant flow or continuous flow of water where the concentration of the tested substance in the water is measured and kept constant through continual addition of the substance to maintain a stable exposure concentration.

**no-observed-effect level (NOEL)** The highest dose or concentration in a toxicity test that results in no statistically significant observed effect in the exposed organisms compared with control organisms.

**oligotrophic** Refers to aquatic environments that have low levels of nutrients and low rates of productivity. Opposite of eutrophic.

**pelagic** (1) Living in the water column of a body of water and having no close association with the bottom substrate. (2) Term applied to organisms of the plankton and nekton that inhabit the open water of a sea or lake.

**probable effect level (PEL)** The concentration of a chemical above which adverse biological effects are expected to occur frequently.

**provisional guidelines** For sediment guidelines: a guideline that has been adopted from another jurisdiction because existing data are insufficient to meet the CCME requirements for guideline derivation.

**receptor/critical receptor** The entity (e.g., person, organism, population, community, or ecosystem) that might be adversely affected by contact with, or exposure to, a substance of concern.

**reference concentration (RC)** A level of a chemical in the tissues of an aquatic prey species (i.e., invertebrates, fish) that is to be consumed by mammalian or avian wildlife. The RC is based on a tolerable daily intake (TDI) and is expected to protect against variation in food

ingestion rates and body weights of consumers. The RC for a given species should be applied to the highest trophic level at which it feeds.

**relative organ weight** Weight of an organ (e.g., liver) adjusted for the total body weight of the organism, allowing comparison of organ weights among individuals of different sizes.

**risk** The probability that a defined undesired effect, such as injury, disease, or death, will result from a specific event, such as a human action, a natural catastrophe, or an exposure to a substance.

**runoff** The portion of the total precipitation on an area that flows into stream channels. Water from surface runoff does not enter the soil. Water from groundwater runoff or seepage flow enters the soil before reaching the stream.

**safety factor** See uncertainty factor.

**sand** A soil or sediment particle between 0.05 and 2 mm in equivalent diameter.

**secondary toxicity** Toxicity that arises not from the direct ingestion a toxicant or food spiked with a toxicant, but rather that which manifests in one organism after consuming another, previously contaminated organism (or parts thereof) such that the toxicant has been subjected to the metabolic processes of the latter while still alive. See also toxicity.

**short-term exposure** Exposure to a contaminant in a medium for a time period that is small compared to the life span of the test organism. Exposure is usually severe enough to rapidly induce an effect. Often referred to as an acute exposure. Absolute definitions for short-term exposure vary from study to study.

**silt** A soil or sediment particle between 0.002 and 0.05 mm in equivalent diameter.

**site-specific objective** Numerical concentration or narrative statement that has been established to maintain and protect a designated resource use at a specified site by taking into consideration site-specific characteristics.

**solubility** The maximum concentration of a substance that will dissolve in a solvent.

**solvent** In aquatic bioassays, an agent (other than water) in which the test chemical is mixed to make it miscible with dilution water before distribution to test chambers.

**sorption** A surface phenomenon that may be either absorption or adsorption, or a combination of

the two. The term is often used when the specific mechanism is not known.

**species** Generally regarded as a group of organisms that resemble each other to a greater degree than their resemblance to members of other groups and that form a reproductively isolated unit that will not normally breed with members of other groups.

**standard** A legally enforceable numerical limit or narrative statement, such as in a regulation, statute, contract, or other legally binding document, that has been adopted from a criterion, guideline, or objective

**static system** An exposure system for aquatic toxicity tests in which the test chambers contain solutions of the test material or control water that are not usually changed during the test. Depending upon conditions, a static system may or may not be in equilibrium.

**sublethal** Below the level that causes death.

**survival time** The time interval between initial exposure of an organism to a harmful parameter and death.

**synergism** A phenomenon in which the toxicity of a mixture of chemicals is greater than that which would be expected from a simple summation of the toxicities of the individual chemicals.

**teratogen** An agent that increases the incidence of congenital malformations.

**teratogenicity** The ability of a chemical to change the normal development processes of an unborn organism, resulting in permanent alterations in the biochemical, physiological, or anatomical functions of the organism.

**threshold** The concentration or dose of a chemical below which the resulting effects cease to be perceptible.

**threshold effect concentration (TEC)** The concentration of a chemical below which adverse effects are expected to occur rarely and above which adverse effects may be expected.

**threshold effect level (TEL)** The concentration of a chemical below which adverse effects are expected to occur rarely.

**tissue residue** The concentration of a foreign chemical or substance measured in the tissue of aquatic biota such as fish, shellfish, invertebrates, and aquatic plants, normally expressed on a

whole-body wet-weight basis.

**tolerable daily intake (TDI)** The level of daily chemical exposure that an organism can sustain with no expected adverse effects. A tolerable daily intake can only be determined for chemicals with threshold effects (i.e., noncarcinogens). It can be expressed as the geometric mean of the LOEL and NOEL, which may be then divided by an uncertainty factor.

**tolerance** The ability of an organism to withstand a given environmental condition for an indefinitely long period of time without dying.

**toxic** Causing or having the potential to cause adverse effects to organisms or populations.

**toxic equivalency or equivalent (TEQ)** See toxic equivalent factor (TEF).

**toxic equivalent factor (TEF)** A relative potency value of a particular PCDD, PCDF, or PCB congener compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin ($T_4$CDD) based on the results of several *in vivo* and *in vitro* studies. TEFs have been derived for a variety of organisms and end-points. Those employed here were derived by the World Health Organisation (WHO) in 1998. Relative potencies of individual congeners (concentration x TEF) may be summed to give a $T_4$CDD toxic equivalent concentration (TEQ). Alternatively, a TEQ may be estimated by comparing the potency of a sample extract to induce enzyme activity (e.g., EROD) to that of a known concentration of 2,3,7,8-TCDD in a cell culture bioassay (e.g., H4IIE).

**toxicant** Agent or material capable of producing an adverse response (effect) in a biological system, seriously injuring structure or function, or producing death.

**toxicity** The inherent potential or capacity of a material to cause adverse effects in a living organism. See also secondary toxicity.

**toxicity test** The means by which the toxicity of a chemical or other material is determined. Toxicity tests are used to measure the degree of response produced by exposure to a specific level of stimulus or concentration of chemical.

**uncertainty factor** A number used to provide an extra margin of safety beyond the known or estimated sensitivities of organisms. Often applied when sufficient information about the toxicity, particularly the chronic toxicity, of a substance is not well known.

**uptake** A process by which substances are absorbed and incorporated into a living organism.

**volatilization** (1) A process by which a substance goes from liquid state to vapour state. (2) A process by which a substance enters the vapour phase.

**wildlife** In reference to tissue residue guidelines, wildlife encompasses mammalian, avian, reptilian, and amphibian species that consume aquatic biota.

## ABBREVIATIONS

### Biological and Chemical Terms

Analytical Methods

| | |
|---|---|
| GC–ECD | gas chromatography with electron capture detection |
| GC-HRMS | gas chromatography-high resolution mass spectrometry |
| GC-MS | gas chromatography-mass spectrometry |
| GC-MSMS | gas chromatography-tandem mass spectrometry |
| GC-NCI | gas chromatography with negative ion chemical ionization detection |

Bioaccumulation

| | |
|---|---|
| BAF | bioaccumulation factor |
| BCF | bioconcentration factor |
| BMF | biomagnification factor |
| BSAF | biota-sediment accumulation factor |
| BI | bioavailability index |

Biochemical Terms

| | |
|---|---|
| *Ah* | aryl hydrocarbon |
| AHH | aryl hydrocarbon hydrolase |
| CYP1A1 | cytochrome P-450-1A1 |
| EROD | ethoxyresorufin *O*-deethylase |
| H4IIE | rat hepatoma cell bioassay |
| MFO | mixed function oxidase |
| RLT | remodulated lightning trout bioassay |

Chemical Names and Terms

| | |
|---|---|
| AVS | acid volatile sulphide |
| BKME | bleached kraft pulp mill effluents |
| Cl | chlorine |
| TBKME | bio-treated bleached kraft pulp mill effluents |
| DDT | 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane |
| DDE | 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethene |
| I-TEF | international toxic equivalency factor |
| PCB | polychlorinated biphenyl |
| PBDDs | polybrominated dibenzo-*p*-dioxins |
| PBDFs | polybrominated dibenzofurans |
| PBBs | polybrominated biphenyls |
| PAH | Polycyclic aromatic hydrocarbons |
| PCDD | polychlorinated dibenzo-*p*-dioxins |
| PCDF | polychlorinated dibenzofurans |
| PCDD/F | PCDD and PCDF |
| $T_4CDD$ | 2,3,7,8-tetrachlorodibenzo-*p*-dioxin |
| $T_4CDF$ | 2,3,7,8-tetrachlorodibenzofuran |
| TEF | 2,3,7,8-TCDD toxic equivalency factor |
| TEQ | 2,3,7,8-TCDD toxic equivalent |
| total TEQ | TEQ based on concentrations of both PCDD/Fs and PCBs |

Chemical Properties

| | |
|---|---|
| log $K_{ow}$ | logarithm of the octanol/water partition coefficient |
| log $K_{oc}$ | logarithm of the organic carbon/water partition coefficient |
| $K_p$ | sediment/water partition coefficient |
| $f_{oc}$ | fraction of organic carbon |
| DOC | dissolved organic carbon |
| TOC | total organic carbon |

Measuring Toxic Effects

| | |
|---|---|
| BEDS | Biological Effects Database for Sediments |
| $EC_{50}$ | effective concentration to 50% of the test population |
| $ED_{50}$ | effective dose to 50% of the test $LD_{50}$ lethal dose to 50% of the test population |
| ELS | early life stage |
| ip | intraperitoneal |
| $LC_{50}$ | lethal concentration to 50% of the test population |
| LOEL | lowest observed effect level |
| MTR | maximum tolerable risk level |
| NOEL | no observed effect level |
| PEL | probable effect level |
| TEC | threshold effect concentration |
| TEL | threshold effect level |

Weights

| | |
|---|---|
| BW or bw | body weight |
| DW or dw | dry weight |
| LW | liver weight |
| RLW | relative liver weight |
| ww | wet weight |

| | |
|---|---|
| EQG | environmental quality guideline |

Water

| | |
|---|---|
| BCF | bioconcentration factor |
| LOEL | lowest observed effect level |
| TEC | threshold effect concentration |
| TRB-EqPA | tissue residue based equilibrium partitioning approach |
| WQG | water quality guideline |

Sediment

| | |
|---|---|
| COA | co-occurrence approach |
| BSAF | biota-sediment accumulation factor |
| EqPA | equilibrium partitioning approach |
| ISQG | interim sediment quality guideline |
| NSTP | National Status and Trends Program |
| PEL | probable effect level |
| SBA | sediment background approach |
| SQAV | sediment quality assessment value |
| SQG | sediment quality guideline |
| SSBA | spiked sediment bioassay approach |
| SSTT | spiked-sediment toxicity test |
| TEL | threshold effect level |
| TRB-EqPA | tissue residue-based equilibrium partitioning approach |

**Guideline Derivation Terms**

Tissue

| | |
|---|---|
| FI | food intake |
| LOEL | lowest observed effect level |
| NOEL | no observed effect level |
| RC | reference concentration |
| TDI | tolerable daily intake |
| TRG | tissue residue guideline |
| UF | uncertainty or safety factor |

**Organizations**

| | |
|---|---|
| BCMOE | British Columbia Ministry of Environment |
| BCMELP | British Columbia Ministry of Environment, Lands, and Parks |
| CCME | Canadian Council of Ministers of the Environment |
| CCMS | Committee on the Challenges of Modern Society |
| CCREM | Canadian Council of Resource and Environment Ministers |
| CFI | Crestbrook Forest Industries Ltd. |
| CIELP | Canadian Institute for Environmental Law and Policy |
| CPPA | Canadian Pulp and Paper Association |
| CWS | Canadian Wildlife Service |
| EC | Environment Canada |
| ECEH | European Centre for Environmental Health |
| HWC | Health and Welfare Canada |
| IJC | International Joint Commission |
| IUPAC | International Union of Pure and Applied Chemistry |
| MEF | Ministère de l'Environnement et Faune |
| MENVIQ | Ministère de l'Environnement du Québec |
| NATO | North Atlantic Treaty Organization |
| NRCC | National Research Council of Canada |
| NYSDEC | New York State Department of Environmental Conservation |
| NWF | National Wildlife Federation |
| NWRI | Nation Water Research Institute |
| OMOE | Ontario Ministry of the Environment |
| SAIC | Science Application International Corporation |
| SRC | Syracuse Research Corporation |
| U.S. EPA | United States Environmental Protection Agency |
| WDE | Washington Department of Ecology |
| WHO | World Health Organization |

**Time**

| | |
|---|---|
| d | day |
| h | hour |
| wk | week |
| a | annum (year) |

# 1. INTRODUCTION

Canadian environmental quality guidelines are developed by the Canadian Council of Ministers of the Environment (CCME) using formal protocols (i.e., CCME 1991a, 1995, 1997) to provide a consistent, scientifically defensible approach for assessing and managing toxic substances in the environment. These guidelines are intended for use by Canadian provincial, territorial, and federal agencies as well as private/corporate stakeholders to assess environmental quality problems and to manage competing uses of resources. Canadian environmental quality guidelines for dioxins and furans are numerical concentrations in various media (water, sediment, aquatic biota) that are recommended to protect, enhance, and restore designated uses of the environment. These concentrations provide benchmarks for the interpretation of environmental monitoring data and serve as the scientific basis for determining interim management objectives and performance indicators to measure progress in virtual elimination strategies. These national numerical environmental quality guidelines are important tools in comprehensive ecosystem management but they are not intended to preclude the need for site-specific considerations and approaches. Thus, it should be noted that the use of the guidelines will require consideration of local conditions.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), commonly known as dioxins and furans, respectively, are planar tricyclic aromatic compounds. As a class of compounds, PCDDs and PCDFs (henceforth abbreviated as PCDD/Fs) are considered 'toxic' substances as defined in Section 11 of the *Canadian Environmental Protection Act*. Additionally, they meet the specifications for Track 1 substances because they are toxic, persistent, bioaccumulative, and concentrations in the environment primarily result from human activities; and as such they are slated for virtual elimination from the environment under the Toxic Substance Management Policy (Environment Canada 1997). In recognition of these characteristics, the CCME requisitioned its Task Group on Water Quality Guidelines to prepare Canadian Environmental Quality Guidelines (EQGs) for PCDD/Fs.

The environmental quality guidelines for PCDD/Fs developed in this document include water quality guidelines (WQGs) for the protection of freshwater and marine/estuarine aquatic biota, sediment quality guidelines (SQGs) for the protection of freshwater and marine/estuarine aquatic biota and tissue residue guidelines (TRGs) for the protection of wildlife consumers of freshwater and marine/estuarine aquatic biota. This report also summarizes information on: physical and chemical properties, production and uses, sources and pathways for entrance into the environment, environmental concentrations, fate and behaviour, bioaccumulation, and relevant toxicological data of dioxins and furans.

The Canadian Environmental Quality Guidelines developed herein, and their supporting information, will contribute to the scientific basis for the development of ambient Canada-Wide Standards (CWSs) for dioxins and furans. Canada Wide Standards are developed under the Canada-Wide Environmental Standards Sub-Agreement of the *Canada-Wide Accord on Environmental Harmonization.*

# 2. IDENTITY, PROPERTIES AND ANALYSIS

## 2.1 Identity and Nomenclature

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are highly persistent compounds which have a strong tendency to accumulate in biological tissues and a strong affinity for sediments (Government of Canada 1990). Both PCDDs and PCDFs are planar tricyclic aromatic compounds with similar properties (WHO 1989). There are a total of 75 PCDD and 135 PCDF congeners, the most studied and most toxic of which is 2,3,7,8,-tetrachloro-*p*-dibenzodioxin ($T_4$CDD) (Government of Canada 1990). This report focuses on the 17 PCDD/F congeners that have chlorine atoms attached at the lateral positions 2,3,7, and 8 (see Figure 1; Table 1). Physical and chemical properties of these congeners are summarized in Tables 1 to 6.

## 2.2 Toxic Equivalency Factors (TEFs) and Toxic Equivalents (TEQs) - Overview

PCDD/Fs are usually released into the environment as complex mixtures (Government of Canada 1990). Like the most toxic congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin ($T_4$CDD), the 16 other PCDD/Fs studied herein are believed to elicit toxic effects through the aryl hydrocarbon (*Ah*) receptor (see Section 7.1). Their relative toxicities, however, may differ by several orders of magnitude (O'Brien 1990). In addition, some polychlorinated biphenyls (PCBs), namely those having a coplanar configuration such as the mono-ortho and non-ortho substituted isomers, exhibit a mode of toxic action similar to that of $T_4$CDD. In order to assess the risk that complex mixtures of dioxin-like compounds pose to aquatic biota or wildlife consumers of aquatic biota, and to facilitate comparisons among environmental samples, the toxic potency of the mixture must be determined and expressed in a common 'currency' (i.e., comparable units). Due to similarities in their mode of action, the toxic potency of a mixture of PCDD/Fs and coplanar

PCBs may be expressed as an equivalent concentration of $T_4$CDD by accounting for the potencies of the individual $T_4$CDD-like compounds relative to that of $T_4$CDD (Walker and Peterson 1994a).

To account for the differences in potency when assessing the potential consequences of the concentrations and cumulative effects of PCDDs, PCDFs, PCBs, and other related halogenated aromatic compounds that occur in complex mixtures in the environment, it has been proposed that toxic equivalency factors (TEFs) be applied to adjust for differences in enzyme induction potency and the potential for toxic effects (Safe 1990; Coulston and Kolbye 1994). TEFs are fractional potencies used in exposure assessments to estimate $T_4$CDD toxic equivalents (TEQs) in organisms based on the sum of TEF-normalized planar halogenated hydrocarbon concentrations (Tillitt et al. 1996). This method is based on the determination of the relative toxicities of dioxin-like substances in relation to that of $T_4$CDD. TEFs are assigned to each chemical based on the results of both *in vivo* and *in vitro* studies that commonly measure enzymatic induction in mammals (rats) and mammalian cell (rat hepatocyte) lines. Typically, concentrations of individual PCDD, PCDF, and PCB congeners are quantified and their relative potencies (concentration x TEF) are summed, giving a TEQ concentration. Mammalian-based TEFs developed by Safe (1990 or 1994) or International TEFs (I-TEFs) developed by NATO/CCMS (1988) are most frequently used to calculate a TEQ, although some TEF values are available for fish and birds (Table 7). The most recent TEFs for fish, mammalian, and avian receptors were developed in 1998 under the Environmental Health and Safety program of WHO and were chosen for use in this document (see Table 8; van den Berg et al. 1998). TEF based TEQs and bioassay based TEQs are discussed further under Section 7.3.

## 2.3 Analysis

There are a wide variety of methods used to analyze dioxins and furans in environmental

samples. Regardless of which technique or environmental matrix is considered, the analytical process involves three general steps: sample extraction, sample clean-up, and quantification. There are no validated protocols, though some methods are preferred depending on the type of sample and its size, data required, and budget. In general, analysis of PCDD/Fs is difficult, time and labour intensive, and costly. A detailed evaluation of these methods is beyond the scope of this work. The following descriptions are taken from the published analytical methods of the Ontario Ministry of the Environment (Laboratory Services Branch), but typify the methods that are commonly employed to quantify dioxins and furans in water, sediment, and tissue samples.

### 2.3.1 *Water*

Prior to analysis, water samples (minimum one litre) should be stored in amber glass bottles with a Teflon- or aluminum foil-lined lid, previously solvent rinsed and baked. Samples should be transported and stored in the dark at cool temperatures (<8°C) for a maximum of 30 d prior to analysis to minimize chemical reactions and/or breakdown. Known quantities of isotopically labelled PCDD/Fs are added to each sample to serve as internal quantitation standards. Samples are then filtered using a solid phase adsorption disk; water soluble PCDD/F congeners are absorbed by the disk while particle bound congeners are trapped on the disk. PCDD/Fs are extracted from the disk and particles with a toluene/ethanol mixture. Extracts are then cleaned using a multi-stage chromatographic clean-up procedure. Additional clean-up using carbon chromatography may be necessary prior to final analysis if the sample is contaminated with other polychlorinated aromatics or polychlorinated diphenyl ethers that are not removed by the open-column chromatograghic technique. Moreover, heavily contaminated samples may cause difficulties in the sample clean-up procedure. Such samples are acid washed prior to open-column chromatography and/or carbon chromatography. The final extract is analyzed by Gas Chromatography-High Resolution Mass Spectrometry (GC-HRMS) or Gas Chromatography-Tandem Mass Spectrometry (GC-MSMS). Detection limits are generally 1 $pg \cdot L^{-1}$ (OMOE

1997a).

### 2.3.2 *Sediment*

Sediment samples should be stored in amber glass bottles with a Teflon- or aluminum foil-lined lid, previously solvent rinsed and baked. Although samples may be stored for an indefinite period of time prior to analysis, exposure to excessive light and temperatures should be avoided to minimize chemical reactions and/or breakdown. Samples (minimum 10 g dry weight equivalent) are dried, ground and homogenized. Known quantities of isotopically labelled PCDD/Fs are added to each sample to serve as internal quantitation standards. A Soxhlet apparatus with toluene is used to extract the PCDD/F congeners. Extracts are then cleaned using a multi-stage chromatographic clean-up procedure. If the sample is contaminated with other polychlorinated aromatics or polychlorinated diphenyl ethers, additional clean-up using liquid/solid chromatography or carbon chromatography, respectively, may be necessary prior to final analysis. Moreover, heavily contaminated samples may cause difficulties in the sample clean-up procedure. Such samples are acid washed prior to open-column chromatography and/or carbon chromatography. The final extract is analyzed by GC-HRMS or GC-MSMS. Detection limits are generally 1 $ng \cdot kg^{-1}$ dw (OMOE 1997b).

### 2.3.3 *Tissue*

Tissue samples should be stored in amber glass bottles with a Teflon- or aluminum foil-lined lid, previously solvent rinsed and baked. Whole fish fillets should packed in solvent rinsed aluminum foil. Samples may be stored in a freezer at a maximum temperature of -4°C for an indefinite period prior to analysis. Thawed samples (2-20 g ww) are homogenized by mechanical grinding of the tissue. Known quantities of isotopically labelled PCDD/Fs are added to each sample to serve as internal quantitation standards. This sub-sample is digested overnight

with concentrated hydrochloric acid and then extracted with hexane. The extract is passed through a glass column containing anhydrous sodium sulphate and sulphuric acid-modified silica (OMOE 1997c). Alternatively, the homogenized sample is ground with anhydrous sodium sulphate into free flowing mixture. This mixture is then transferred to a chromatography column and spiked with isotopically labelled PCDDs and PCDFs (Huestis et al. 1995). The samples are then eluted with methylene chloride. Albeit no overnight digestion with hydrochloric acid is required in this latter case, lipids must be removed in a separate gel permeation chromatography step. Some analytical labs routinely perform carbon chromatography clean-up on the extracts before instrumental analysis while others employ it only if the sample is contaminated with polychlorinated diphenyl ethers. The final extract is concentrated with a rotary evaporator and subsequently fractionated using HPLC. As for other media, the final extract is analyzed by GC-HRMS or GC-MSMS. This analytical method is optimised for fish tissue, however, it is also applicable to other aquatic animals. Detection limits are generally 1 $ng \cdot kg^{-1}$ (OMOE 1997c).

The practice of lipid-normalization simplifies sample extraction, analytical procedures, and interpretation of analytical results. For instance, for whole body measurements, large fish would not always need to be homogenized prior to analysis especially if the ratio of the lipid and analyte content in the organ of interest to that of the whole fish were measured first. Therefore, whole body concentrations could be estimated in subsequent samples using this ratio. Moreover, composites of small samples, as well as tissue subsamples, could be used and compared directly on a lipid weight basis because the greatest degree of variation among such samples would likely be due to differing lipid contents. Nevertheless, the efficacy of lipid-normalization of wet or dry weight-based analyte concentrations may be affected by the extraction method used to estimate lipid content. Randall et al. (1991) evaluated four methods for determining the lipid content of fish and invertebrate tissues and noted that, depending on the extraction solvent, the results varied by a factor as high as 3.5. While no one solvent proved to be superior, the authors recommended that a standard protocol should be adopted to ensure comparability of data. An overview of the pertinent literature shows that the lipid fraction may be reasonably reproducible

since most laboratories follow standard protocols for PCDD/F extraction from tissue. Typically, Soxhlet or column extractions with dichloromethane:hexane (1:1) are performed for lipid extraction. Extractions using these nonpolar solvents are fairly reproducible within a laboratory and yield mainly triglycerides (D. Muir, Freshwater Institute, Dept. of Fisheries and Oceans, pers. com. 1997).

### 2.3.4 *Variations on Analysis*

Recently, the Centre d'Expertise en Analyse Environnementale du Québec of the Ministère de l'Environnement et de la Faune developed a new protocol for the analysis of PCDD/Fs, PCBs, and PAHs in soil, sediment, water (1-50 L), and tissue samples entitled, "Ultra-Trace Multi-Parameters Method". This procedure involves a new adsorbent, a silver loaded alumino-silicate gel (AgAlSi) that has the capability to completely separate PAHs from PCDD/Fs and PCBs. The PCDD/F - PCB fraction is further separated on an alumina column to give three fractions: PCBs, mono-ortho and planar PCBs, and PCDD/Fs. Because the extract is not split in two or three before purification, and because all of the quantifications are done by HRMS (PCDD/Fs, planar PCBs, PAHs) or MS/MS (other PCB congeners), detection limits are lower than more standard approaches; those for water, sedment, and tissue are reported as 0.01 - 0.05 $pg \cdot L^{-1}$, 0.1 - 1 $pg \cdot g^{-1}$ and 0.1 - 1 $pg \cdot g^{-1}$, respectively (C. Brochu, MEF, pers. com. 1998/99).

# 3. SOURCES AND PATHWAYS FOR ENTERING THE AQUATIC ENVIRONMENT

PCDD/Fs are not intentionally produced, and there are no known uses of these compounds (WHO 1989; Fiedler et al. 1990). They are involuntary impurities formed as a result of anthropogenic activities, including combustion, chemical production, metal processing, and pulp and paper production. Each of these source categories will be examined in detail below. Natural sources of PCDD/Fs include forest fires, volcanic activity, and other forms of natural combustion (Hicks and McColl 1995).

An inventory of sources of PCDD/Fs in Canada is currently being prepared by Environment Canada (Environment Canada 1998a). Data compiled in a draft of this report (October 1998) forms the basis of the review provided in this section. Quantitative data from earlier studies are also presented in order to illustrate the temporal trends in PCDD/F release in Canada (Table 9).

## 3.1 Combustion Sources

Atmospheric release from combustion sources is the largest contributor of PCDD/Fs to the Canadian environment (Environment Canada 1998a). Airborne PCDD/Fs, in both the vapour and particulate phases, may be transported long distances and deposited through wet or dry deposition (Steer et al. 1990; Bobet et al. 1990). As such, both aquatic and terrestrial ecosystems may become contaminated with PCDD/Fs via this pathway. Combustion sources include municipal waste incinerators; biomedical waste incinerators; coal-fired utility boilers; oil and gas burning; fuel wood burning; forest fires; and cigarette smoking, among others (Sheffield 1985). Combustion of many carbon-based materials with minute quantities of chlorine, organic chlorine compounds or inorganic chlorides leads to the production of dioxins and furans in limited amounts (Hicks and McColl 1995; Rappe et al. 1987). PCDDs released from combustion sources consist generally of the less toxic $H_7CDD$ and OCDD congeners (Sheffield 1985; Fiedler

et al. 1990). This congener profile, however, is often reversed for PCDFs, such that the smaller $T_4$CDF and $P_5$CDF congeners are the predominant forms (Fiedler et al. 1990).

In Canada, between 1990 and 1997, an 18% reduction in atmospheric releases of PCDD/Fs was observed (from 353 to 290 g TEQ·a$^{-1}$) (Environment Canada 1998a). By 1999, a 25% reduction in atmospheric releases is projected compared to 1990 levels. It is anticipated this reduction will be achieved due to facility upgrades and/or closures (Figure 2).

### 3.1.1 *Waste Incinerators*

An incinerator can be described as a reaction chamber for a complex mixture of atomic and molecular species. The elevated temperature and high concentration of carbon, oxygen, and chlorine species may provide suitable conditions for the formation of dioxins and furans (Hicks and McColl 1995). Effective destruction of dioxins and furans can only be achieved under adequate incineration conditions (Hutzinger et al. 1985). Generally, the incineration process is not 100% effective and a small fraction of the organics present may not be destroyed (Hicks and McColl 1995).

There are several types of incinerators operating in Canada. Municipal waste incinerators, hazardous waste incinerators, and biomedical waste incinerators all contribute in varying degrees to the release of PCDD/Fs to the Canadian environment.

Municipal waste incinerators are the most significant source of atmospheric emissions of PCDD/Fs in Canada, releasing 152 g TEQ·a$^{-1}$ to the environment in 1997 (Environment Canada 1998a). The Canadian inventory for 1997 indicates that releases of PCDD/Fs from nine large municipal waste incinerators in Canada were approximately 66.9 g TEQ·a$^{-1}$ (Environment Canada 1998a). The majority of these emissions (92%) come from one facility in Quebec. Renovations to this facility are expected to reduce emissions to 5.1 g TEQ·a$^{-1}$ by 1999

(Environment Canada 1998a). Smaller municipal incinerators in British Columbia (9.4 g TEQ·a$^{-1}$ in 1997) and teepee municipal solid waste burners in Newfoundland (75.4 g TEQ·a$^{-1}$ in 1997) also contribute to the total releases from this sector (Environment Canada 1998a). Rappe et al. (1987) reported that the major source of chlorine in municipal solid waste incinerators is plastic material (e.g., PVC). Direct evidence for the conversion of PVC to PCDDs and PCDFs has been reported by Marklund et al. (1986).

Hazardous waste incinerators are of public concern, especially those where PCB are being burned. Typical hazardous waste streams consist of contaminated process wastewater, residues from chemical process industries, paint residues, chemical spill cleanups, solids, soils, oils, and others. The congener profile for PCDD/Fs from hazardous waste incinerators is similar to those reported from municipal waste incinerators (Rappe et al. 1984; Marklund et al. 1986). In 1998, there were four hazardous waste incineration facilities with five incinerators operating in Canada. Approximately 1.3 g TEQ·a$^{-1}$ of PCDD/Fs were released from these four facilities in 1997 (Environment Canada 1998a).

Biomedical waste incineration is a common operation performed at hospitals and has also been identified as a source of dioxins and furans. Biomedical waste incinerators destroy medical waste that includes all hospital waste except for corpses and body parts. The estimated PCDD/F emissions from hospital incinerators in Canada were 8.3 g TEQ·a$^{-1}$ in 1995 (Environment Canada 1998a). Several hospital incinerators have been shut down in the past few years thus reducing total PCDD/PCDF releases to approximately 2.5 g TEQ·a$^{-1}$ in 1997 (Environment Canada 1998a).

### 3.1.2 *Oil, Coal, and Gas Burning and Refining*

There are conflicting and limited data regarding dioxin formation by the burning of coal.

Studies that examine the detection relationship of emission concentrations with other variables such as the chlorine content of coal and combustion conditions are needed (Hutzinger et al. 1985). There is some indication that by burning sulfur-rich fuel, such as coal, along with waste in municipal incinerators, the production of dioxins and furans can be significantly reduced. Emissions of PCDD/Fs from coal-fired power plants (Kimble and Gross 1980), wood stoves (Nestrick and Lamparski 1982), and peat burning (Marklund et al. 1986) seem to be very low when calculated in $m^3$ flue gas. Nonetheless, the very high flow rates and large number of units could make a significant total contribution to PCDD/F emissions.

An inventory of PCDD/F releases by the petroleum industry is unavailable. Results of a study conducted in Ontario indicate that PCDD/Fs are present in stack emissions and wastewater effluents from various petroleum refineries (Thompson et al. 1990). PCDD/F concentrations as high as 480 and 1500 $pg \cdot L^{-1}$, respectively, were measured in internal effluent streams from these facilities. In stack emissions, concentrations of total PCDD were as high as 9 $ng \cdot m^{-3}$, while concentrations of total PCDF were as high as 210 $ng \cdot m^{-3}$ (Thompson et al. 1990). No quantitative data is reported in the draft Canada inventory at this time (Environment Canada 1998a).

Automobiles are known contributors to PCDD/F emissions in Canada. Ballschmiter et al. (1986) identified a series of PCDD/Fs in used motor oil from automobiles. They suggested chlorinated additives in motor oil or in gasoline as possible sources for the PCDD/Fs, but no quantitative data were reported. Fuel combustion emissions from a number of different types of vehicles were estimated using U.S. EPA emission factors. Diesel engines are thought to be responsible for approximately 8.7 g $TEQ \cdot a^{-1}$, while gasoline engines are estimated to contribute 0.1 g $TEQ \cdot a^{-1}$ (Environment Canada 1998a).

### 3.1.3 *Cement Kilns*

PCDD/F emissions from cement kilns have been quantified from 15 of the 28 kilns in Canada. The results suggest that emissions from this sector amount to approximately 2.8 g TEQ·a$^{-1}$ (Environment Canada 1998a). Cement kilns typically burn alternative fuels, such as tires, waste oil, coal, coke, solvents and bunker oil.

### 3.1.4 *Wood Burning*

Residential burning of untreated wood has been identified as a source of PCDD/Fs. Samples of bottom ash and chimney ash were collected from two wood burning stoves, one open fireplace, and from out-of-doors open-air burning. Although only untreated wood was burned, PCDD/Fs were detected in all samples (Clement et al. 1985). Large differences in total PCDD/F levels and relative congener amounts were observed between samples, although within-congener patterns were very similar and in some cases resembled the patterns detected in municipal incinerator fly ash. Residential wood combustion in Canada contributed 35.7 g TEQ·a$^{-1}$ to the Canadian environment in 1997 (Environment Canada 1998a) (Table 10).

## 3.2 Chemical Production

PCDD/Fs are impurities in a wide variety of commercial chemicals used in Canada. Trace quantities of PCDD/Fs occur in many chemicals, mainly as a result of high temperatures and chlorinated solvents that are used in manufacturing processes. Substances known to be contaminated with PCDDs and/or PCDFs include chlorophenols (e.g., pentachlorophenol), various pesticides (e.g., 2,4-D and 2,4,5-T), 1,2,4-trichlorobenzene, hexachlorobenzene, tetrachlorobenzoquinones, askarels [polychlorinated biphenyl (PCB) mixtures used in electric transformers], and perchloroethylene (Fiedler et al. 1990). In Canada, the total PCDD/F released in liquid effluents for the chemical production sector was 3.7 g TEQ·a$^{-1}$ in 1990 (Environment

Canada 1998a). By 1997, the majority of these plants had modified their processes resulting in a total release of 0.01 g TEQ·a$^{-1}$ into effluents (Environment Canada 1998a).

Pentachlorophenol is a wood preservative known to contain trace amounts of PCDD/F impurities (CCME 1997). Many of the historic uses of pentachlorophenol in Canada have been restricted in recent years through the Pest Control Products Act. Nonetheless, CPI Product Profiles (1991) reported that 1000 tonnes of PCP were used at wood preservation facilities in 1990. In British Columbia, chlorophenols in mixtures of creosote are used mainly in wood preservation and protection, especially in railway ties, trestles, and utility and telecommunication poles. This use pattern creates a potential for wood poles and railway ties to contaminate adjacent soil with dioxins and furans (Wan and Van Oostdam 1995). Garrett and Shrimpton (1988) reported that accidental spills and runoff from lumber treatment and storage sites can result in the contamination of nearby surface waters. Spills, runoff, and disposal of PCP-contaminated wastes at these and other sites have the potential to contaminate soils and groundwater in the vicinity of the operation. Sheffield (1985) estimated that in 1983, releases of total PCDD/F amounted to >2.4 kg·a$^{-1}$ from processes using PCP. In 1997, the twelve wood preservation plants currently operating in Canada released a total of ~1.8 g TEQ·a$^{-1}$ (Environment Canada 1998a).

## 3.3 Metal Production

In Canada, metal processing contributed approximately 53 g TEQ·a$^{-1}$ of PCDD/Fs in 1990 (Environment Canada 1998a). This amount reflects the combined estimates for iron manufacturing (sintering), steel manufacturing (electric arc furnaces and foundries), and base metal smelting.

There are two metal sintering plants located in Ontario one of which slated to be closed in June 1999. Anticipated release of PCDD/Fs from this activity in 1999 is 23.5 g TEQ·a$^{-1}$

(Environment Canada 1998a) compared to 43 g TEQ·a$^{-1}$ in 1990. Measured PCDD/Fs released from a base metals smelting plant in Quebec was determined to be approximately 0.1 g TEQ·a$^{-1}$ in 1997 (Environment Canada 1998a).

In the steel mills, a high portion of the alloys or stainless steel were recycled. The recycled material was contaminated by PVC or polychlorinated paraffin. Environment Canada (1998) reported that electric arc furnaces were the fifth largest source of atmospheric release of PCDD/PCDF though releases have increased since 1990.

### 3.4 Pulp and Paper Mills

Pulp and paper mills may emit PCDD/Fs into the environment by a number of routes. Significant quantities of the most toxic PCDD/F congeners were discharged directly to freshwater, estuarine, and marine systems (Government of Canada 1990). The use of unlined settling basins to trap particulate matter in liquid effluents at some pulp and paper mills, may also result in the contamination of groundwater. In addition, disposal of sludge from settling basins and clarifiers in sanitary landfills may result in contamination of soils, and groundwater and/or surface water if significant leaching takes place. A wide variety of pulp and paper products are known to be contaminated with PCDD/Fs (Berry et al. 1989; Canadian Pulp and Paper Association 1989), and their ultimate disposal (i.e., burning or landfilling) could result in additional releases to the environment (Sheffield 1985). Finished bleached paper products that are known to contain PCDD/Fs include newsprint, laboratory filter paper, coffee filters, cosmetic tissue, recycled scrap paper, milk cartons and other bleached paperboard containers (Table 11; Beck et al. 1989; Beck et al. 1990; Kitunen and Salkinoja-Salonen 1989; Ryan et al. 1991; Safe 1991). The formation of PCDD/Fs in the pulp and paper industry is associated with chlorine bleaching in which the natural phenolic constituents of wood pulp (e.g., lignin) are chlorinated to yield chlorinated phenolic compounds which are precursors of PCDD/Fs (Safe 1991).

Prior to 1992, PCDD/Fs released from this sector in liquid effluents amounted to approximately 450 g TEQ·$a^{-1}$. Environment Canada estimated the dioxin and furan discharges from bleached kraft pulp mill effluents in 1988/89 to be 280g of TEQs. British Columbia has a unique problem in that pulp wood is typically transported down the Pacific coast to pulp and paper mills. This results in large quantities of salt (NaCl) being adsorbed by the wood. The chlorine in the salt then facilitates the production of PCDD/Fs during processing.

Kraft liquour boilers are used by the pulp and paper industry and have been identified as a potential source of PCDD/Fs. Two kraft liquour boilers have been tested, one by the industry in British Columbia and one by Environment Canada in Quebec. The concentration of PCDD/Fs exiting the stacks ranged from 0.004 and 0.008 ng·$m^{-3}$. It has been estimated that kraft liquour boilers in Canada released a total of 1.4 g TEQ·$a^{-1}$ in 1997 (Environment Canada 1998a).

# 4. FATE AND PERSISTENCE IN AQUATIC SYSTEMS

## 4.1 Water

Each of the 17 priority PCDD/F congeners considered in the present evaluation has extremely low solubility in water (i.e., $<0.5\ \mu g \cdot L^{-1}$) (Table 3). Additional information on the physicochemical properties of these substances, such as log $K_{ow}$ and log $K_{oc}$ suggest that PCDD/Fs are likely to form associations with both dissolved and particulate organic matter upon entry into aquatic ecosystems (Tables 5 and 6; Webster et al. 1986). Because they are hydrophobic, the majority of PCDD/Fs released into aquatic systems are likely to become associated with suspended or bed sediments, or the tissues of aquatic organisms (Corbet et al. 1988).

Photolysis may be a significant degradation process for aqueous PCDD/Fs under certain circumstances. Choudhry and Webster (1986) estimated the photolytic half-lives of six PCDDs in water bodies at latitude 40°N using phototransformation data gathered from a photochemical reactor (313 nm), molecular extinction coefficients, and solar intensity data published for different seasons at the given latitude. The results of this study indicate that 1,2,7,8-TCDD, 1,3,6,8-TCDD, 1,2,3,4,7-PCDD, 1,2,3,4,6,7,8-HCDD, and OCDD degrade most rapidly during the summer, with calculated half-lives of 0.3, 1.8, 15.2, 6.27, 47.3, and 17.9 d, respectively. Half-lives were longest in winter (0.84 to 156 d), and intermediate in fall (0.53 to 88 d) and in spring (0.35 to 56 d). Authors did not provide reasons for the variability, but it is presumably related to seasonal differences in solar intensity. A slightly longer a photolytic half-life of 6.3 d has been also reported for 1,3,6,8-TCDD in pond water (Corbet et al. 1988). These values are similar to the photolytic half-life of $T_4CDD$ in water (27 to 81 h) that was estimated from information on degradation rates in distilled water solutions (SRC 1989b; Howard 1991). Together, these data indicate that aqueous photolysis is likely an important fate process in

shallow water, especially for the lower chlorinated PCDDs, during periods of high incident solar radiation. It is important to note that the photolysis of higher chlorinated PCDD congeners produces lower chlorinated forms (Miller et al. 1989). Therefore, releases of the less toxic $H_7CDD$ and OCDD congeners could be significant if photolysis in water resulted in the formation of the more toxic $T_4CDD$ and $P_5CDD$ congeners.

Biodegradation is considered to be a relatively minor environmental fate process in water (NRCC 1981). In a test of 100 microbial strains, only five of these demonstrated any ability to degrade $T_4CDD$ (Matsumura and Benezet 1973). An unacclimated aqueous aerobic biodegradation half-life was estimated to be between 1.15 and 1.62 years for $T_4CDD$, and using the same data, the anaerobic biodegradation half-life was estimated to be between 4.58 and 6.45 years (Howard 1991).

The physicochemical properties of PCDD/Fs suggest that volatilization is likely to be an insignificant fate process under most circumstances. Using available data on vapour pressure, the estimated half-life for $T_4CDD$ volatilization from a pond would be 5.5 years, whereas, it would be 12 years from a lake; the difference likely due to the former having a greater surface area - volume ratio than the latter (OMOE 1985). As the vapour pressures of PCDD/Fs decrease with increasing chlorination, longer half-lives would be predicted for the higher molecular weight PCDD/Fs (Hutzinger et al. 1985). In accordance with these predictions, the volatilization of 1,3,6,8-TCDD was found to be minimal during the first 34 days after it was added to outdoor pools (Corbet et al. 1988). Somewhat surprisingly, however, was the detection of significant quantities of radiolabelled-OCDD in air samples collected above the surfaces of outdoor ponds treated with 340 and 680 $ng \cdot L^{-1}$ (Marcheterre et al. 1985).

No data were located on the fate of PCDD/Fs in groundwater. The half-life of $T_4CDD$ in groundwater was estimated to be between 2.3 and 3.2 years (SRC 1989a; Howard 1991). This estimate was based on aerobic biodegradation rates observed for this substance in a soil column

study in which aerobic groundwater was continuously percolated through quartz sand (Kappeler and Wuhrmann 1978).

Adsorption to organic matter is the most important fate process for PCDD/Fs released into surface waters; both OCDD and 1,3,6,8-TCDD rapidly partitioned to suspended particulate matter and dissolved organic matter in mesocosm studies conducted in northern Ontario (Servos et al. 1992a). This information is consistent with other data indicating that the concentrations of PCDDs in the water phase (including both the dissolved and particulate forms) decline rapidly in these types of test systems. For example, aqueous half-lives for 1,3,6,8-TCDD in outdoor pools were estimated to be in the order of 14 to 28.5 h (Corbet et al. 1988). Similarly, aqueous half-lives of 2.6 and 4.0 d for $T_4$CDD and OCDD, respectively, were reported in large lake enclosures in northern Ontario (Servos et al. 1992a). Therefore, adsorption to organic matter results in the rapid removal of PCDDs from the aqueous phase of surface waters. In relation to toxicity, this fate process is important because it decreases the bioavailability of PCDD/Fs in the water column, or the fraction of chemical that is available for uptake by aquatic organisms (Suffet et al. 1994).

Another reason that adsorption to organic matter is an important aquatic fate process is that the PCDD/Fs bound to organic matter are then deposited onto the bed sediments, making sediments a major sink for PCDD/Fs that enter the water column. For example, after 34 days, 34 to 80% of the 1,3,6,8-TCDD added to test systems were associated with sediments (Corbet et al. 1988). Similarly, sharp increases in the concentration of $T_4$CDD in sediments were observed within days of adding this substance to water in a model aquatic ecosystem (Tsushimoto et al. 1982). It was also found that when 1,3,6,8-TCDD is added to sediment/water systems in laboratory studies, it partitions almost entirely into the sediment phase (Muir et al. 1985a).

## 4.2 Sediment

PCDD/Fs have high affinities for aquatic sediment and, as such, these substances may accumulate to significant levels in this medium (Czuczwa and Hites 1986). Little information was found on photolysis, hydrolysis, or microbial degradation of PCDD/Fs in aquatic sediments. The results of several laboratory incubation studies suggest that these fate processes are minor. For example, after 675 d under stable aerobic conditions, 80% of the radiolabelled 1,3,6,8-TCDD added to water/sediment system was still present in pond and lake sediments as the parent compound (Muir et al. 1985a). Similarly, it was estimated that only 1 to 4% of the $T_4CDD$ added to laboratory sediment/water systems was degraded over a 588 d period (Ward and Matsumura 1978).

The fate of sediment-associated PCDDs is more complex in test systems that are designed to simulate aquatic ecosystems. In a model aquatic ecosystem (roughly 185 $m^3$) that consisted of water, sediment (4.1% organic matter), two aquatic macrophytes (*Elodea nuttali* and *Ceratophyllum demersum*), and fathead minnows (*Pimephales promelas*), the addition of 3.4 mCi of $^{14}C$-$T_4CDD$ (initial measured concentration of 53.7 $ng \cdot L^{-1}$) resulted in a rapid increase in its concentration in the sediments (up to 2700 $ng \cdot kg^{-1}$ ww) within the first four days of the study (Tsushimoto et al. 1982). Within 50 days, the concentration of $T_4CDD$ in sediment dropped to 500 $ng \cdot kg^{-1}$ww, and to 97 $ng \cdot kg^{-1}$ww within 365 days. During that period, the concentrations of $T_4CDD$ in the macrophytes and fish increased to over 2000 $ng \cdot kg^{-1}$ ww, indicating significant transfer of this substance into biological tissues, primarily from the sediments. The macrophyte-associated $T_4CDD$ accounted for more than 85% of the $T_4CDD$-radioactivity remaining after 365 days in this mesocosm. After 750 days, sediment-associated $T_4CDD$ accounted for virtually all of the remaining $T_4CDD$-radioactivity measured in the system. It was unclear if this radioactivity was associated with increased levels of $T_4CDD$ bound to fish faeces; however, the results demonstrate that $T_4CDD$ may undergo complex cycling between the abiotic and biotic components of the ecosystem.

In a similar study, Servos et al. (1992a) investigated the fate of 1,3,6,8-TCDD and OCDD in large (40 $m^3$) enclosures in Lake 304 of the experimental lakes area of north-western Ontario. These mesocosms consisted of water (2 m deep), sediment (25.4% organic carbon), and the benthic and planktonic organisms that typically occur in the lake. The results of this study indicate that these substances are very stable in bed sediments. After 720 days, sediment-associated 1,3,6,8-TCDD accounted for 57% of the $^{14}C$ initially added to the mesocosm. Likewise, OCDD in sediment accounted for 55% of the radiolabelled-OCDD originally added to the test system. In shallow outdoor pools, however, only a small proportion (7.9-17.7 %) of the 1,3,6,8-TCDD originally added to the test system (which included water, sediment, rooted plants, and duckweed) was associated with sediments after 426 days (Corbet et al. 1988). These investigators suggested that photolysis, uptake and biotransformation by plants, and degradation in sediments were responsible for the significant losses of 1,3,6,8-TCDD observed during the study.

PCDDs may persist in natural freshwater and marine sediments for long periods (OMOE 1985). For example, low levels of the higher chlorinated PCDD congeners were found in lake sediments that were 300 to 1000 years old (Jansson et al. 1987). Likewise, significant quantities of 1,2,3,4,6,7,9-HCDD (52 $ng \cdot kg^{-1}$) and OCDD (320 $ng \cdot kg^{-1}$) were detected in deep sediments from an inland sea in Japan, estimated to be approximately 8120 years old (Hashimoto et al. 1990). These data indicate that PCDDs may be very stable in sediments below the biologically-active layer (i.e., top 5-15 cm), particularly in areas with high sedimentation rates.

Several biological processes may redistribute PCDD/Fs within bed sediments and reintroduce these substances into the water column. Many benthic organisms (e.g., tubificid worms, clams, polychaetes) burrow to significant depths in bed sediments, resulting in the mixing of surficial sediments with deeper materials. For example, tubificid worms can mix lake sediments to a depth of 10 cm and release contaminants directly into the water column (Fisher et al. 1980;

Karickhoff and Morris 1985). Similarly, clams can burrow up to 20-30 cm into bed sediments (Lee 1991). Polychaetes are even more effective, burrowing to depths of 50 cm and accounting for more than 90% of the movement of hydrophobic organic contaminants in certain locations (Karickhoff and Morris 1985; Eadie et al. 1988). Historical deposits of PCDD/Fs now found in deep sediments, may therefore be redistributed to less contaminated surficial sediments through bioturbation. Uptake by, and death of, aquatic organisms may represent an important cycling process for sediment-associated PCDD/Fs (Tsushimoto et al. 1982).

In summary, PCDDs are very stable in sediments and, therefore, tend to persist for extended periods in this environmental matrix; fate processes such as photolysis, hydrolysis, or microbial degradation of PCDD/Fs are believed to be insignificant. As such, PCDD/Fs that are associated with bed sediments may represent long term sources to the aquatic food web (Kuehl et al. 1987b; Muir 1988).

### 4.3 Aquatic Biota

Aquatic organisms may be exposed to PCDD/Fs through direct contact with water and sediment, and through the consumption of contaminated food. The relative importance of each of these exposure routes is likely to differ significantly between species and even between various life stages. Batterman et al. (1989) evaluated the importance of each of these exposure routes in lake trout (*Salvelinus namaycush*) and concluded that bioaccumulation occurs primarily through the consumption of contaminated prey species. For carp (*Cyprinus carpio*), however, PCDD/F contaminated bed sediments may represent the primary exposure route (van der Weiden et al. 1989b). Direct exposure to PCDD-contaminated water has also resulted in bioconcentration of these substances in the tissues of rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (Muir et al. 1985b,c). One study on guppies (*Poecilia reticulata*) under controlled laboratory conditions suggests that for this species water exposure may be a more important uptake route

for PCDD/Fs than ingestion of contaminated food (Loonen et al. 1993).

PCDD/Fs are highly hydrophobic substances and, as such, are readily accumulated in the tissues of aquatic biota. Yet, PCDD/Fs are atypical of chlorinated aromatic hydrocarbons with comparable hydrophobicity (i.e., polychlorinated benzenes and biphenyls) because their uptake rates are significantly reduced relative to these other compounds (Grimwood and Dobbs 1995). As such, the bioaccumulation of these substances cannot be accurately predicted from their physicochemical properties (such as log $K_{ow}$; Table 5). Rather, the rate and extent of PCDD/F accumulation in aquatic organisms is dependent on the position of the chlorine substitution, the size of the molecule, and elimination rates (Adams et al. 1986; Kuehl et al. 1986; Opperhuizen and Sijm 1990; Sijm et al. 1990). In a study on the uptake, bioconcentration, and depuration of six PCDD congeners in rainbow trout and fathead minnows, high uptake rates for the $T_4$CDD and $P_5$CDD congeners were reported, while the higher chlorinated congeners were taken up more slowly (Muir et al. 1985b; c). Likewise, Muir et al. (1990) observed high assimilation efficiencies (41 to 44%) when rainbow trout were administered 2,3,4,7,8-PCDD in their diet, while lower efficiencies were observed for the $H_6$CDD (37%) and $H_7$CDD (13%) congeners (Muir and Yarechewski 1988). Assimilation efficiencies for five other congeners, including 2,3,4,7,8-$P_5$CDF (22%), $H_7$CDD (8%), $H_7$CDF (5%), OCDD (2%), and OCDF (0 to 1%) were relatively low in the guppy (Clark and Mackay 1991; Loonen et al. 1991).

Following uptake, PCDD/Fs are distributed throughout the tissues of aquatic organisms with preferential accumulation in tissues with high lipid content (i.e., >4%; Sijm et al. 1990). In rainbow trout, the muscle, skin, liver and intestine were major storage sites for $T_4$CDD, $P_5$CDD, and $P_5$CDF (Sijm et al. 1990). Greater than 90% of the $T_4$CDD extracted from rainbow trout, following a 13 week exposure period, was found in the visceral fat, carcass, skin, pyloric caeca, and all fatty tissues (Kleeman et al. 1986a). Similarly, nearly 80% of the total body burden of $T_4$CDD in yellow perch was contained in the carcass (including head, fins, bones, and cartilaginous material) and visceral fat (Kleeman et al. 1986b).

Limited information is available on the biotransformation of PCDD/Fs in aquatic organisms. Kleeman et al. (1986b) found that of the $T_4CDD$ administered in the diet of yellow perch, between 1 and 3% was transformed, with the gallbladder containing almost all of the $T_4CDD$ metabolites. One of these metabolites was identified as a glucuronide conjugate. These data are supported by the results of a more recent study, in which biotransformation of $T_4CDF$ to a glucuronide conjugate in rainbow trout was observed (Muir et al. 1992a).

PCDD/Fs tend to be relatively persistent in the tissues of aquatic biota. A depuration half-life of 18 wk was reported for yellow perch administered a single oral dose of $T_4CDD$ (Kleeman et al. 1986b). Similarly, the depuration half-lives for $T_4CDF$ and $P_5CDF$ in rainbow trout were approximately 10 wk (Muir 1991). In contrast, it was reported that 1,3,6,8-TCDD and $P_5CDD$ were eliminated rapidly from rainbow trout fry and fathead minnows, with average half-lives of 2.6 and 3 d, respectively (Muir et al. 1985b). The half-lives of the higher chlorinated $H_6CDD$ and $H_7CDD$ were longer in both rainbow trout (16 d) and fathead minnows (20 d) than the lower chlorinated congeners. These data suggest that elimination rates are species, life stage, and congener specific.

# 5. BIOCONCENTRATION, BIOACCUMULATION, AND BIOMAGNIFICATION

Bioaccumulation and bioconcentration are sometimes used interchangeably to describe the accumulation of organic contaminants in biota; however, the two terms have different meanings and are dependent on the routes by which a contaminant is accumulated. Bioconcentration refers to the direct uptake of compounds from water across the gills and retention in the tissues of aquatic organisms, whereas bioaccumulation involves the biological uptake of substances from all compartments, including water, food, and sediment (Branson et al. 1985, Muir et al. 1992b). Biomagnification refers to the increase in tissue concentrations of accumulated chemicals from one trophic level to the next (i.e., organisms contain higher concentrations of the substance than their food sources).

## 5.1 Bioconcentration/Bioaccumulation from Water

Bioconcentration data are generally reported as bioconcentration factors (BCFs) which are defined as the contaminant concentration measured in the biota divided by the contaminant concentration in the water. Ideally, the BCF should reflect a steady-state condition, where the BCF remains constant over time and is described by Oliver and Niimi (1985) in the following equation:

$$BCF = C_b/C_w = k_1/k_2$$

where:

BCF = bioconcentration factor;

$C_b$ = chemical concentration in the organism ($mg \cdot kg^{-1}$ ww);

$C_w$ = chemical concentration in the water ($mg \cdot L^{-1}$);

$k_1$ = uptake rate constant; and

$k_2$ = elimination rate constant.

According the Toxic Substances Management Policy, a substance is considered bioaccumulative if its BCF in fish is greater than 5000 (Government of Canada/Environment Canada 1995).

The distinction between bioaccumulation and bioconcentration is sometimes lost in the literature. Many research efforts have been devoted to measuring PCDD/F accumulation in fish from both processes but frequently values reported as bioconcentration factors are in fact, bioaccumulation factors (BAFs), because they include contributions to total body burdens from food and sediment as well as water exposure pathways. For example, 'BCFs' derived from field data are not considered true BCFs because presumably organisms in the field would be ingesting food containing PCDD/Fs in conjunction with uptake across the gills. It is also possible that these organisms may be taking up PCDD/Fs through contact or ingestion of contaminated sediment.

The duration of exposure, levels of dissolved organic carbon, concentration of the congener in the water, and species and life stage of the test organism may affect the BCF. A summary of published, laboratory derived, BCFs reflecting only uptake from water is provided in Table 12. The BCFs listed in Table 12 have been converted to lipid-based values wherever possible to facilitate comparisons between different life stages and species; conversion to a lipid basis rather than a wet weight basis removes some of the variability associated with different species of aquatic organisms and different life stages. Table 12 is restricted to information that concerns 2,3,7,8-substituted congeners as these are the most toxic forms and are generally the only congeners reported in tissue samples of higher organisms (van den Berg et al. 1994). Information pertaining to invertebrate species is not included because studies containing data on the uptake of PCDD/Fs were invariably confounded by the contribution to total exposure by contaminated foodstuffs.

From the available data it appears that BCFs at steady-state or estimated at steady-state are

highest for $T_4CDD$ with $BCFs_{lipid}$ ranging from 50 900 for rainbow trout (Servos et al. 1989) to 5 100 000 for medaka, *Oryzias latipes* (Schmieder et al. 1995). A geometric mean $BCF_{lipid}$ of 175 245 was calculated for $T_4CDD$ from the available steady-state (or estimates thereof) data for resident fish species. Penta-chlorinated dioxins and furans also bioconcentrate to relatively high levels; the $BCFs_{lipid}$ for 1,2,3,7,8-PCDF/1,2,3,4,8-PCDF, 2,3,4,7,8-PCDF, and 1,2,3,7,8-PCDD were 21 400, 240 000, and 331 000, respectively in guppies (Loonen et al. 1994a). The $BCFs_{lipid}$ at steady-state (or estimates thereof) for hexachlorinated congeners are slightly lower, indicating that they do not accumulate to as great a degree as do pentachlorinated dioxins and furans. For the hexachlorinated dioxins and furans, the $BCFs_{lipid}$ ranged from 11 360 for 1,2,3,4,7,8-HCDD in rainbow trout (Servos et al. 1989) to 174 000 for 1,2,3,6,7,8-HCDD and 1,2,3,6,7,8-HCDF in guppies (Loonen et al. 1994a). $T_4CDF$ accumulates to an even lesser degree with $BCFs_{lipid}$ ranging from 21 400 in guppies (Loonen et al. 1994a) to 120 980 in rainbow trout (Mehrle et al. 1988). Higher-chlorinated congeners (i.e., hepta- and octa-chlorinated) show the least accumulation with a $BCF_{lipid}$ at a minimum of 2 710 in fathead minnow for 1,2,3,4,6,7,8-HCDD (Muir et al. 1985b). High $BCFs_{lipid}$ have been measured for the latter congener (560 540 and 635 780 in rainbow trout; Servos et al. 1989) in relation to the maximum recorded for the heptachlorinated furans and octachlorinated congeners ($BCF_{lipid}$ = 42 700 for 1,2,3,4,6,7,8-HCDF in guppies; Loonen et al. 1994a).

The molecular size and/or the solubility characteristics of the molecule may explain the decrease in bioaccumulation observed with the higher chlorinated congeners (i.e., the hexa-, hepta- and octachlorinated dioxins and furans). It has been proposed that membrane permeation by hydrophobic compounds that have an effective cross section larger than 0.95 nm would be minimized, thus preventing uptake via the gills (Opperhuizen and Sijm 1990). This idea has been substantiated by other authors who found that the low BCFs of hexa- and octa-chlorinated congeners were primarily due to steric and solubility factors affecting membrane permeation, rather than to low bioavailability caused by binding with organic carbon (Muir et al. 1985b,c; Gobas and Schrap 1990). Strong binding with organic carbon is associated with compounds that

have low water solubility, and has been put forth as another explanation for the low accumulation of the higher chlorinated congeners observed in aquatic organisms. Low water solubility may also account for low bioaccumulation because compounds with low water solubility require long times for steady state conditions to be attained. Molecular size, water solubility, and sorption to organic matter are related issues that may affect the uptake of the higher chlorinated dioxins and furans.

To summarize, all 2,3,7,8-substituted PCDD/Fs are readily concentrated in the tissues of aquatic organisms, with the higher chlorinated PCDD/Fs bioaccumulated to a lesser degree than the lower chlorinated congeners. The $BCFs_{lipid}$ recorded for $T_4CDD$ were the highest of all congeners and, therefore, may be used as a conservative measure to estimate uptake of PCDD/Fs from water only (not including uptake from food or sediment exposure). A geometric mean $BCF_{lipid}$ for $T_4CDD$ of 175 245 was calculated from the available steady-state (or estimates thereof) data for resident fish species, reflects species differences, differential exposure conditions (i.e., variable water concentrations, dissolved organic carbon levels in the water etc.), and various life stages. This number, however, reflects only freshwater conditions as data pertaining to marine/estuarine environments were not located.

## 5.2 Bioaccumulation from Sediments

Bioaccumulation via the food chain, originating in the organic fraction of the sediments, is an important path of uptake of PCDD/Fs by aquatic organisms. In a mesocosm study, it was demonstrated that emerging aquatic insects alone are capable of removing a small, but biologically significant, portion of $T_4CDF$ from sediments each year (Fairchild et al. 1992). Aquatic insects are a significant food source for many aquatic and terrestrial predators. As such, they are likely an important link between sediment-associated contaminants and food chains. Hence, the sediments, and in particular the organic fraction, serve as a reservoir for uptake of

PCDD/Fs by benthic organisms at the base of the food chain.

While BCFs describe the relationship between the concentration of a compound in an organism and the level of the compound in the surrounding aqueous environment, attempts have also been made to link sediment concentrations of PCDD/Fs with concentrations in invertebrates and fish (Carey et al. 1990; Cook et al. 1991; Muir et al. 1992a, b). Due to the affinity of PCDD/Fs for the fatty tissues in organisms and for the organic fraction of sediments, a biota-sediment accumulation factor (BSAF), sometimes called a bioavailability index (BI), is commonly used to characterize the tissue/sediment relationship. This BSAF is an accumulation factor (concentration in organism on a lipid (ww) basis divided by the concentration in sediment organic carbon) that assumes equilibrium of the contaminant between the two compartments. Adjusting or normalizing for lipid content of the organism and for organic carbon content of the sediment generally reduces data variability. For example, no statistically significant relationships were found between concentrations in sediment and concentrations in suckers and whitefish unless the data were normalized (Muir et al. 1992a). Similarly, Lake et al. (1990) found that normalizing BSAFs for PCBs in molluscs and polychaetes also reduced variability, as did Carey et al. (1990) for $T_4$CDD in several Lake Ontario fish species.

Field and laboratory BSAFs for PCDD/Fs for freshwater and marine/estuarine organisms are summarized in Table 13. Due to the limited available data, it is difficult to assess differences in BSAFs for $T_4$CDD between freshwater and marine/estuarine environments. In general, it can be concluded that fish consuming detritus at the sediment-water interface (i.e., suckers, carp, BSAFs = 0.14 to 0.96; Mah et al. 1989; Muir et al. 1992a), fish preying on filter-feeding insects (i.e., whitefish, BSAFs = 0.28 – 1.88; Mah et al. 1989 as reported in Muir et al. 1992a, Muir et al. 1992a), and invertebrates residing in or on the sediments (i.e., worms, clams, BSAFs = 0.14 to 0.93; Rubinstein et al. 1990; Schrock et al. 1997) have the highest accumulation factors; pelagic species such as lake (*Salvelinus namaycush*) and brook trout (*Salvelinus fontinalis)* and smallmouth bass (*Micropterus dolomieui*), which have relatively much less contact with the

sediments, have comparably lower BSAFs for $T_4CDD$ (0.03 to 0.11; Carey et al. 1990, Cook 1990). Field-derived BSAFs of 1 to 3 were estimated for four 2,3,7,8 substituted PCDD/Fs in lake trout while values for other congeners were well <1 (Niimi 1996). Other studies reviewed by this author suggest little difference between BSAFs for lateral (2,3,7,8 substituted) and non-lateral substituted PCDD/Fs (Niimi 1996).

Clearly, site-specific conditions such as the concentration of the compound in the sediment and percent organic matter will influence the magnitude of field-derived BSAFs. The type of tissue (e.g., liver, muscle etc.) in which the compound is measured may also affect the BSAF. Moreover, it is difficult to ascertain whether the assumption of steady-state conditions has been met for field-derived data, therefore some of the variability in the data may be due to violating this assumption. Finally, for much of the data contained in Table 13, sediment concentrations of the compounds in question were not analytically detectable so best estimates were calculated using detection limits of the compound in sediment. While there is a certain amount of variability inherent to BSAFs, these measures provide valuable estimates of uptake of PCDD/Fs by aquatic organisms in relation to exposure to contaminated sediments. An overall BSAF for $T_4CDD$ was calculated separately for the freshwater and marine/estuarine environments. Laboratory and field-derived data were combined for each type of environment (i.e., freshwater versus marine/estuarine ) and BSAFs calculated based on non-detectable concentrations (set at detection limits) in the sediment were included in the determination of overall BSAFs because the degree of variability in the BSAFs appeared to outweigh the error associated with a particular method of estimation (i.e., field vs. lab, detectable levels in sediments vs. non-detectable levels). Data for guppies and eels (*Anguilla anguilla*) were not included in the calculation, as these species do not occur in Canadian waters. Conversely, the BSAFs for organisms from Rice Creek, Florida were included in the calculation of the overall BSAF for marine/estuarine data because the species sampled are found in Canadian waters. The geometric mean BSAF for $T_4CDD$, representing an overall BSAF for freshwater environments calculated from the available data is 0.30, while the overall BSAF for marine/estuarine environments is 0.14.

## 5.3 Bioaccumulation and Biomagnification

While it is generally accepted that the food chain is the major source of lipophilic halogenated aromatic hydrocarbons for wildlife, the trophodynamic behaviour of PCDD/Fs is poorly understood. PCDD/Fs appear anomalous compared to other halogenated aromatic hydrocarbons in that they do not tend to biomagnify to an appreciable degree (Grimwood and Dobbs 1995; Niimi 1996). For example, biomagnification factors (BMFs) for PCDD/Fs, although variable, were typically <1 over several trophic levels in a Lake Ontario food chain. In contrast, BMFs for PCBs in the same study often exceeded 100 (Niimi 1996). This author suggested that dietary adsorption efficiencies for PCDD/Fs, which are consistently lower and more variable than those for PCBs, and high elimination efficiencies could largely account for the lower BMFs observed for PCDD/Fs.

Low BMFs have also been reported for mammalian and avian species that consume aquatic biota. For instance, BMFs for mink (*Mustela vison*), normalized to an average consumption of 0.22 g of food·(g of mink)$^{-1}$·day$^{-1}$ and based on a 40% carp diet, were 6.4 to 74.2 for PCDDs and <1 to 75.8 for PCDFs (Tillitt et al. 1996). In general, the BMFs increased with the degree of chlorination (Tillitt et al. 1996). Estimated BMFs for herring gulls (*Larus argentatus*) consuming alewife (*Alosa pseudoharengus*) are 32, 20, 14, 6.6, and 1.3 for $T_4$CDD, 1,2,3,6,7,8-HCDD, 1,2,3,7,8-PCDD, 2,3,4,7,8-PCDF, and 2,3,7,8-TCDF, respectively (Braune and Norstrom 1989). The liver of these birds contained 5 to 55% of the total body burden. In addition, these toxicants were transferred from mother to egg with the highest concentration in the eggs reported as 83 ng·kg$^{-1}$ ww for $T_4$CDD (Braune and Norstrom 1989). As chlorination increased, maternal transfer of PCDD/Fs to the eggs decreased despite increased PCDD/F retention in the mothers' livers (Braune and Norstrom 1989). Once hatched, chicks may also uptake PCDD/Fs; an uptake rate for 1,2,3,6,7,8-HCDF of 0.001 ng·d$^{-1}$ has been reported for

Forster's tern (*Sterna forsteri*) chicks, with greater concentrations of 1,2,3,6,7,8-HCDF, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HCDD, and 1,2,3,4,6,7,8-HCDD occurring in the chicks than in the eggs from the same nest (Ankley et al. 1993). In a study of the accumulation of PCDD/Fs in eider ducks (*Somateria mollissima*), it was found that only 10% of the total PCCD/Fs were retained in the tissues of the ducks, although, of the most toxic congeners, 57% were retained in the tissues (Broman et al. 1992). This study also found that although the total concentration of PCDD/Fs decreased with increasing trophic level, the most toxic congeners tended to biomagnify.

In harbour porpoises (*Phocoena phocoena*) on the Dutch coast, PCDDs and PCDFs were found in low concentrations of up to 4.6 and 2.1 pg·kg$^{-1}$ fat, respectively (van Scheppingen et al. 1996). The authors found that PCDD/Fs concentrations in porpoises were lower than those found in herring from the same area. Moreover, PCDD/Fs in porpoises contributed ≤0.5% of the total TEQ (PCDD/Fs and PCBs). The authors concluded that PCDD/Fs do not biomagnify in this food chain. Similarly, harbour porpoises off the coast of California did not have any detectable concentrations of PCCD/Fs, although, in the same study, harbour porpoises off the coast of British Columbia had concentrations of 1,2,3,6,7,8-HCDD of up to 128 ng·kg$^{-1}$ ww blubber and 2,3,7,8-TCDF of up to 43 ng·kg$^{-1}$ ww blubber (Jarman et al. 1996). The authors attributed this difference to the greater number of pulp mills in British Columbia than in California. The relative contribution of bioconcentrated and bioaccumulated PCDD/Fs were not addressed in this study.

In a study of trophic transfer from Canadian ringed seals (*Phoca hispida*) to polar bears (*Thalarctos maritimus*), all seal samples and all but one polar bear had detectable TCDD at concentrations ranging from 2 to 37 ng·kg$^{-1}$ blubber. All ringed seal samples contained 2,3,7,8-TCDF at levels of 2 to 7 ng·kg$^{-1}$ blubber, but TCDF was not found in any bear sample. No other PCDF congeners were found in seals or bears. 1,2,3,6,7,8-HCDD was found in only two of the ringed seal samples (8 and 9 ng·kg$^{-1}$ blubber) and none of the polar bear samples. OCDD

concentrations were found in four of the seals and all but two of the polar bear samples up to 43 $ng \cdot kg^{-1}$ blubber (Table 14). The authors concluded that TCDD, OCDD, and TCDF do not biomagnify from seal to bear (Norstrom et al. 1990).

The above data is based on a limited literature search; therefore, no concrete conclusions regarding bioaccumulation or biomagnification of PCDD/Fs can be made at this time. From the data presented here and below in Section 6.5, it appears that PCDD/Fs can accumulate to appreciable levels in animals but that biomagnification does not occur; the greatest BMF reported were 32 and 76 for herring gulls and mink, respectively (Braune and Norstrom 1989; Tillitt et al. 1996). The accumulation of PCCD/Fs may still pose a threat to higher organisms. In a hazard assessment study of the effects of TCDD through the food chain, Loonen et al. (1996) concluded that fish-eating birds and mammals were at a greater risk of detrimental effects from the accumulation of $T_4CDD$. The authors found, through back calculation to water concentrations, that the no effect concentrations in water were lower for birds and mammals than for fish and invertebrates (Loonen et al. 1996).

# 6. ENVIRONMENTAL DISTRIBUTION AND LEVELS

Dioxins and furans enter the environment through both natural and anthropogenic sources (see Chapter 3). Though they have never been commercially manufactured, they are produced as unintentional by-products or trace impurities (Fiedler and Hutzinger 1990). Nevertheless, dioxins and furans are widely distributed in environmental media (i.e., air, soil, sediment, water, and biota). As dioxins and furans are very hydrophobic, they tend to concentrate in sediment, soils, and bioaccumulate in aquatic and terrestrial biota (see Chapter 5). There is a relatively large amount of information in the scientific literature regarding PCDD/F concentrations in the Canadian environment. The purpose of the following discussion is to provide an overview of the distribution and levels of PCDD/Fs in Canada. Available data on the levels of PCDD/Fs in the Canadian environment are presented in Tables 14 through 30.

## 6.1 Air

As part of an initiative by the Canadian Council of Resource and Environment Ministers (now the CCME), methodology development for ambient air monitoring for PCDD/F began in 1987 (Steer et al. 1990). Since that time, standardized analytical and sampling methods have been developed and are in use at monitoring stations across Canada (Dann 1998).

Steer et al. (1990) monitored PCDD/F concentrations at three sites in Ontario from 1988-1989 (Table 15). $T_4CDD$ and $T_4CDF$ concentrations ranged from 0.05 – 1 and 0.02 – 1 $pg \cdot m^{-3}$, respectively. The three sampling sites were Toronto Island, Dorset and Windsor, ON. The authors reported that contributing source types (e.g., waste incineration vs. chemical production sources) identified by the variation in specific PCDD/F congeners detected, were substantially different in Windsor than both Toronto Island and Dorset.

Bobet et al. (1990) reported on PCDD/F levels detected in the first year of operation of an ambient air monitoring network in Southwestern Ontario. Sample sites included Windsor and Walpole Island, ON.. Levels of $T_4$CDD and $T_4$CDF were below detection limits at both sites. Other PCDD/F congeners were also detected and reported. The total mean PCDD and PCDF concentrations reported for Windsor were 2.12 and 0.46 pg·m$^{-3}$ respectively. For Walpole Island the total mean PCDD level was 0.51 pg·m$^{-3}$. No PCDF was reported for Walpole Island.

Environment Canada maintains a National Air Pollution Surveillance (NAPS) network that samples ambient air concentrations of PCDD/F among other substances in Canada (Dann 1998). The sampling equipment includes a high-volume sampler with a dry-gas meter or rotary vane meter and a filter-sorbent sampling system to collect particulate plus vapour phase PCDD/F. Daily samples are taken from 34 monitoring sites, but the historic record for monitoring data at most of the sites is limited. $T_4$CDD and $T_4$CDF levels range from 0.004 to 0.19, and 0.001 to 0.05 pg·m$^{-3}$, respectively for 1994-1997. Dann (1998) found that mean TEQ concentrations (based on I-TEFs) across Canada ranged from 0.1 pg·m$^{-3}$ at a site in Toronto to 0.001 pg·m$^{-3}$ at St. Andrews, NB.

## 6.2 Soil

In British Columbia, a monitoring program was used to determine baseline levels of dioxins and furans in an assortment of environmental media not impacted by pulp and paper mills (Van Oostdam and Ward 1995). In all, 53 background soil samples were collected across the province (Table 16) with concentrations ranging from below detection to 0.057 µg TEQ·kg$^{-1}$ dw, and an average of 0.005 µg TEQ·kg$^{-1}$ dw (based in I-TEFs). Background soil samples are believed to be representative of ambient levels in the environment (Van Oostdam and Ward 1995). The CCME remediation criteria for agricultural soils is 0.01 µg TEQs·kg$^{-1}$ dw, and for residential parkland and soils is 1 µg TEQ·kg$^{-1}$ dw, both based in I-TEFs (CCME 1991b). None of the 53 background

soil samples collected exceeded the residential/parkland soil criteria, however, nine of the samples did exceed the agricultural soils criteria (Van Oostdam and Ward 1995). In addition to the background soil samples, 31 samples from areas close to potential contamination sources (termed primary soil samples) and 137 sample from areas directly impacted by the primary source (termed secondary samples) were also collected (Van Oostdam and Ward 1995). The average TEQs (based in I-TEFs) for primary and secondary soil samples in British Columbia were 0.252 and 0.242 $\mu g \cdot kg^{-1}$ dw, respectively. Overall, levels of dioxins and furans in British Columbia are variable. Elevated levels of dioxins and furans in the environment may be due to past and/or present industrial activities either at a particular site or in the proximity (Van Oosdam and Ward 1995).

McLaughlin et al. (1989) reported dioxin and furan levels in soil surrounding a large municipal waste incinerator in Hamilton, ON, in 1983. Fourteen locations, including three control sites were sampled (Table 16). The highest concentration of PCDD/F detected was 3.5 $\mu g \cdot kg^{-1}$ dw for OCDD, from a sample taken 1260 m southwest of the municipal incinerator. A similar value of 3.2 $\mu g$ OCDD$\cdot kg^{-1}$ dw, was detected at one of the urban control sites well removed from the incinerator. Generally, no significant differences in dioxin and furan levels were noted between the soils of the urban control sites and locations immediately surrounding the incinerator.

Chemicals used in wood preservation (e.g., pentachlorophenol and creosote) are known to contain PCDD/F impurities (CCME 1997; Wan and Van Oostdam 1995). Railway ties, trestles, and utility and telecommunication poles are commonly preserved using these chemicals. Soil in contact with preserved wood can become contaminated by PCDD/F. Soil contamination can occur due to chemical exudation, bleeding via gravitational forces, transport through surface runoff and leaching processes of the preservative chemicals and impurities into the soil (Wan and Van Oostdam 1995). In 1990 and 1991, samples taken from farmlands, utility and railway right-of-way (ROW) ditch sediments in the Lower Mainland of British Columbia were tested for PCDD/F contamination. Sediment levels of 3.7 $\mu g \cdot kg^{-1}$ dw PCDD and 0.7 $\mu g \cdot kg^{-1}$ dw PCDF

were detected in farmland ditches (Table 17; Wan and Van Oostdam 1995). Levels of PCDD/F in railway ditch sediments were much higher than the dioxin and furan concentrations detected in farmland ditches (e.g., 18.8 $\mu g \cdot kg^{-1}$ dw PCDD and 6.5 $\mu g \cdot kg^{-1}$ dw PCDF) (Wan and Van Oostdam 1995). The authors concluded that levels of PCDD/F detected in farmland ditches varied according to the location of potential contamination sources, such as railway ties and telecommunication poles (Wan and Van Oostdam 1995).

Baker and Matheson (1981) reported PCDD/F concentrations at two wood preserving sites in Nova Scotia and New Brunswick. The highest concentrations of dioxins and furans were found on the property of the wood preserving plants. The plant in Truro, NS had estimated $H_6CDD$ and $H_7CDD$ concentrations of 10 and 100 $\mu g \cdot kg^{-1}$ dw, respectively. In Newcastle, NB a similar pattern was found. Concentrations of $H_6CDD$ and $H_7CDD$ were estimated at 100 and 1000 $\mu g \cdot kg^{-1}$ dw, respectively, and OCDD was detected at 1500 $\mu g \cdot kg^{-1}$ dw on the wood preserving property in New Brunswick (Table 16).

## 6.3 Water

Canadian surface waters may be contaminated with dioxins and furans from an assortment of environmental media including atmospheric deposition, effluents from pulp and paper mills using chlorine bleaching, chemical manufacturing, waste incineration, petroleum refining, and sewage sludge, among others (Kuehl et al.1987; Hicks and McColl 1995; Sheffield 1985). There are difficulties associated with analyzing ambient water for these substances at ultra-trace levels because they are adsorbed by particulate matter or are rapidly taken up by biota (Fiedler and Hutzinger 1990).

In the spring of 1992, Environment Canada initiated water quality investigations in a 200 km range of the Athabasca River (from Hinton to Whitecourt, AB) under the Northern River Basin

Study (NRBS). PCDD/Fs were detected in the Athabasca River at low levels, some of which continued for 230 km downstream of Hinton. Sampling was performed on the Athabasca River at sites often influenced by pulp mill effluent and/or sewage treatment plants. The 2,3,7,8-homologue was the only $T_4CDD$ detected from Hinton combined effluent (0.35 $pg \cdot L^{-1}$; Table 18). 2,3,7,8-$T_4CDF$ (0.78 $pg \cdot L^{-1}$) was detected in the water from Hinton combined effluent, and four other $T_4CDFs$ were also detected, with 2,4,6,8-$T_4CDF$ (75 $pg \cdot L^{-1}$) being the major component (Crosley 1996).

In 1989, Alberta Environment collected raw and treated drinking water samples from seven municipalities in upstream and downstream locations of existing kraft mills and analyzed these samples for PCDD/Fs (Table 18; Alberta 1991). Most of the samples had PCDD/F concentrations below the instrument detection limit (Alberta 1991).

Public perception surrounding the presence of chlorinated dioxins and furans in bleached kraft mill effluent resulted in concerns with respect to the potential impact of mill effluents on drinking water supplies in Alberta municipalities (Milos 1990). In 1989, a dioxin/furan sampling program was initiated for a few chosen communities downstream of existing pulp mills (Milos 1990). Raw and treated water samples were collected from seven municipalities (Table 18). No dioxins or furans were measured within the limits of the method detection level (detection limit in the range of 6-70 $pg \cdot L^{-1}$ depending upon the congener group) at any of the sites. The authors concluded that there was no evidence of a problem with dioxins or furans in raw or treated drinking waters downstream from existing bleached kraft discharges in Alberta (Milos 1990).

In November, 1988, a directive to close the crab fishery near a pulp mill in Prince Rupert, BC as well as the crab, prawn, and shrimp fisheries in the Howe Sound areas near the Woodfibre and Port Mellon pulp mills was issued by the Federal Department of Fisheries and Oceans (BCMOE 1989). As a precautionary measure for nearby communities, drinking water samples were collected from twelve sites, whose intake sources are downstream of pulp mills effluent

discharges (with the exception of one site - Quesnel sample - which is upstream from the influence of the two Quesnel mills) (Table 18) (BCMOE 1989). The majority of the measured PCDD/F levels were less than the analytical detection limits (BCMOE 1989). In 1989, groundwater samples in British Columbia were collected and analyzed for PCDD/F (Table 18). No dioxin or furan levels were detected (BCMOE 1989).

In 1980-1981, water samples were collected from 13 water treatment plants scattered throughout Ontario to examine levels of PCDD (Table 18). PCDDs were not detected in any of the samples (n=20; DL = 1000 $pg \cdot L^{-1}$; OMOE 1985). In 1980 to 1982, water samples were analyzed for PCDDs from nine water works in Western Lake Ontario. The detection limit for the water samples was 250 $pg \cdot L^{-1}$ $T_4CDD$. A total of 143 sampled were analyzed and none of the water samples exceeded the detection limits for $T_4CDD$ (OMOE 1985).

Precipitation was measured in two sites in Ontario, one rural location (Dorset) and one urban location (Toronto Islands), from 1986-1988 (Tashiro and Clement 1989; Reid et al. 1990). The congener detected most often in the precipitation samples was OCDD (range: 60 to 1200 $pg \cdot L^{-1}$) (Table 19) (Tashiro and Clement 1989). PCDD/F levels at the rural location were generally higher than concentrations received at the urban site (Reid et al. 1990). In Dorset, samples taken in the winter months had the highest PCDD/F concentrations. The authors suggest that the high concentrations in the winter months were due to residential wood burning, which is expected to be more prevalent in rural areas (Reid et al. 1990). Overall, PCDD and PCDF levels in precipitation samples throughout Ontario were very low.

## 6.4 Sediment

### 6.4.1 *Freshwater Sediment*

Trudel (1991) examined levels of PCDD/F in sediment at numerous sites in Ontario and found pulp mill effluent discharges to be a major source of dioxins and furans. Sediments are considered "highly" to "very highly" contaminated when PCDD/F concentrations range from 60–200 to 200-600 ng TEQ·kg$^{-1}$ dw, respectively (Table 20) (Trudel 1991). $T_4$CDD and $T_4$CDF concentrations reached as high as 66 ng·kg$^{-1}$ dw and 1200 ng·kg$^{-1}$ dw (%OC= 4.8), measured downstream from a pulp mill on the Wabigoon River, near Dryden. The reported TEQ (based on I-TEFs) for this site was 208 ng·kg$^{-1}$ dw (Trudel 1991). The highest TEQ (270.4 ng·kg$^{-1}$ dw, %OC=3.26) was found downstream from a pulp mill at Red Rock, Lake Superior (Trudel 1991).

In Québec, the majority of the sediment collected from the area surrounding pulp and paper mills was determined as having "intermediate" PCDD/F contamination levels (<60 ng TEQ·kg$^{-1}$ dw) (I-TEFs; Trudel 1991). The only TEQ level above 60 ng·kg$^{-1}$ dw in Québec (63.9 ng TEQ·kg$^{-1}$ dw; %OC=3.81) was found downstream from a pulp and paper mill in Quévillon River (Trudel 1991). Most sediment samples in New Brunswick and Nova Scotia also fall under the "intermediate" sediment contamination levels category for dioxins and furans, however, sediment contamination levels from Miramichi River, NB were detected at a level of 91 ng $T_4$CDF·kg$^{-1}$ dw, (%OC=8.32), at an upstream location from a pulp and paper mill, and 34 ng $T_4$CDF·kg$^{-1}$ dw, (%OC=2), at a location downstream from a pulp and paper mill. Trudel (1991) also reported elevated concentrations of total $H_7$CDD and OCDD of 250 and 960 ng·kg$^{-1}$ dw, respectively, upstream of Miramichi Pulp and Paper in Newcastle, NB (Trudel 1991).

In British Columbia, bed sediments were collected from many upstream and downstream locations in the vicinity of four pulp and paper mills, and one petroleum refinery (Table 20). Most of the samples collected from the Pine and Peace Rivers upstream and downstream of Fibrelco Pulp Incorporated and the Petro-Canada Products refinery did not contain any PCDD/F residues (Tuominen and Sekela 1992). The bed sediments collected from the Kitimat River

upstream of the Eurocan Pulp and Paper Company and upstream of the lower Fraser River mills had no detectable residues of dioxins or furans, however, sediment samples collected downstream of the lower Fraser River mills contained detectable residues of furans. $T_4CDF$ levels from 19 to 24 $ng \cdot kg^{-1}$ dw, with organic carbon ranging from 1.57% to 1.88%, were detected in sediments collected downstream of Scott Paper Limited, New Westminster, and a similar level of 22 ng $T_4CDF \cdot kg^{-1}$ dw with an organic carbon content of 0.61% was collected downstream of Paperboard Industries Corporation, Burnaby (Tuominen and Sekela 1992).

Background sediment samples are thought to be characteristic of ambient levels of dioxins and furans in the environment (Van Oostdam and Ward 1995). In British Columbia, there were no detectable levels of $T_4CDD$ in background samples of sediments (Table 20). The average TEQ (based in I-TEFs) for background sediment samples was 3.9 $ng \cdot kg^{-1}$ dw, with a range from 0.0 to 24.4 $ng \cdot kg^{-1}$ dw; the TEQ range for secondary sediment samples was from 0.0 to 172 $ng \cdot kg^{-1}$ dw (Van Oostdam and Ward 1995).

Mah et al. (1989) reported concentrations of dioxins and furans in sediments collected in the proximity of ten pulp mills at upstream and downstream locations in the interior of British Columbia, in 1988 (Table 20). At most of the upstream sites, dioxin and furan congeners in bed sediments were below the detection limits, with the exception of the site located on the Fraser River, upstream of the pulp mills at Quesnel and downstream of discharges from pulp mills at Prince George (Mah et al. 1989). Most of the sediment samples collected downstream of the pulp mills contained PCDFs. The highest furan concentration (3168 ng $T_4CDF \cdot kg^{-1}$ dw, %OC=1.2) in bed sediments was collected downstream from Weyerhauser Canada Limited, in the Thompson River. Other similarly high levels of furans were detected downstream of Crestbrook Forest Industries Limited (2217 ng $T_4CDF \cdot kg^{-1}$ dw, %OC=13) and near Fletcher Challenge Canada Limited in Williston Lake (2077 $ng \cdot kg^{-1}$ $T_4CDF$ dw, %OC=10.6) (Mah et al. 1989). The authors concluded that there did not appear to be a significant relationship between

the concentrations of furans measured in sediment in downstream locations and particle size or organic carbon content (Mah et al. 1989).

In 1990, Dwernychuk et al. (1991a, b) conducted sediment quality surveys on the Fraser, Thompson, and Kootenay River systems in British Columbia. PCDD/F concentrations in the Fraser River were variable. The highest $T_4CDF$ concentration in a sample (%OC = 4.6) was 7.8 $ng \cdot kg^{-1}$, with a TEQ (based on I-TEFs) of 3.8 $ng \cdot kg^{-1}$near the mouth of the Fraser River, along the south arm (Table 20; Dwernychuk et al. 1991b). Most of the sediment collected from the upper Fraser River did not contain detectable levels of furans. The highest level of $T_4CDF$ in sediments collected from the lower Thompson River was 42 $ng \cdot kg^{-1}$, (8.4 ng TEQ$\cdot kg^{-1}$; %OC=3.5) at Wallachin station, followed by the next highest concentration of $T_4CDF$ in sediments collected at Spences Bridge in the lower Thompson River, of 8.9 $ng \cdot kg^{-1}$, (3.8 ng TEQ$\cdot kg^{-1}$; %OC=4.4) (Dwernychuk et al. 1991b). At all four monitoring stations on the Thompson River system and all stations along the Fraser River, $T_4CDD$ levels were below the detection limits for sediments (Dwernychuk et al. 1991b). $T_4CDF$ concentrations ranging from below detection to 23 $ng \cdot kg^{-1}$ were measured in bottom sediment samples collected downstream of Crestbrook (Kootenay River system), and no dioxins were detected (Dwernychuk et al. 1991a).

In 1992-1993, suspended sediments from 22 locations in Alberta were analyzed for PCDD/Fs, including ten mainstem Athabasca River sites, five major tributaries, and seven waste effluent sites. Depositional sediments from nine mainstem Athabasca River sites were also sampled (Table 20) (Crosley 1993). Most of the effluent, mainstem, and tributary locations had detected levels of PCDD/F congeners in suspended and depositional sediments. Suspended sediments from effluents at Millar Western Pulp and Slave Lake Pulp, however, contained very low or non-detectable concentrations PCDD/F (Crosley 1993). The highest $T_4CDD$ concentration in effluent influenced suspended sediments was found in the Hinton combined effluent at 24 $ng \cdot kg^{-1}$ ww (Crosley 1993). $T_4CDD$ concentrations in the majority of the tributaries usually had similar or

lower concentrations to the Athabasca River. Total $T_4CDD$ concentrations were generally one order of magnitude lower in depositional sediments than concentrations in suspended sediments (Crosley 1993).

### 6.4.2 *Marine Sediment*

Marine and estuarine sediments in both the Pacific and Atlantic regions of Canada have been monitored for PCDD/Fs (Trudel 1991). The most contaminated areas of British Columbia appear to be Howe Sound and Georgia Strait, with TEQs (based on I-TEFs) of 145 to 2143 and 16.3 to 1398 $ng \cdot kg^{-1}$ dw, respectively (Table 21; Trudel 1991). Lower TEQ levels were evident in Port Alice, British Columbia, with a range between 50 and 96 $ng \cdot kg^{-1}$ dw (Trudel 1991). On the Atlantic coast of Canada, sediment samples were much less contaminated than those on the Pacific coast. The most contaminated site in Nova Scotia, was Port Hawksbury, with a TEQ level of 67.6 $ng \cdot kg^{-1}$ dw (Trudel 1991).

## 6.5 Biota

There are a large number of studies that have determined the levels of PCDD/Fs in Canadian biota. Certain classes of biota have attracted more research and monitoring attention, particularly birds and fish. Scientific names for all common names mentioned in this section are found in Table 22 if available. The data for tissue residue concentrations of PCDD/F are presented in Tables 14 and 23 – 29 and, where possible, include the toxic equivalency (TEQ) reported by the author(s) and the source of the toxic equivalency factors (TEFs) used by the author(s) to derive the TEQ. Ideally, for comparison purposes, the TEQs for all studies would be calculated using standard TEFs. Where congener specific information was available, the TEQs were calculated using WHO 1998 TEFs for fish, mammalian, and avian species and are reported as $TEQ_{fish}$, $TEQ_{mammalian}$, and $TEQ_{avian}$, respectively (van den Berg et al. 1998). Conversion to WHO 1998

TEQs was not always possible as specific congeners were often not reported. In such cases the TEF source used by the author is reported. Polychlorinated biphenyl (PCB) congeners (i.e., those that exhibit a planar configuration such as the mono-ortho and non-ortho substituted isomers) are not included in the TEQ calculations unless otherwise indicated. All tissue concentrations are reported in wet weight (ww) also unless otherwise indicated.

### 6.5.1 *Freshwater and Marine Invertebrates*

There are very little data available on the concentrations of PCDD/F in freshwater invertebrates in Canada (Table 23). Studies by Dwernychuk et al. (1991a; 1993) along the Fraser and Kootenay Rivers in British Columbia examined the PCDD/F concentrations in various benthic macroinvertebrates and crayfish (*Pacificastus* spp.). Both the Fraser and the Kootenay Rivers are impacted by pulp and paper mill effluent. In 1990, the survey of benthic macroinvertebrates in the Kootenay River found that PCDD/F concentrations were higher downstream of a pulp and paper mill than upstream ($TEQ_{fish}$ = 1.6 ng·kg$^{-1}$ and $TEQ_{fish}$ = 18 ng·kg$^{-1}$, respectively). $T_4CDD$ and $T_4CDF$ concentrations ranged from <0.7 to 1000 ng·kg$^{-1}$, respectively. Benthic invertebrates sampled in a side channel of the Kootenay River downstream of the mill had a TEQ level of 127.4 ng·kg$^{-1}$ (Dwernychuk et al. 1991a). Along the Fraser River, a composite sample of crayfish (*Pacificastus* spp.) muscle (n=21) had non-detectable levels of $TEQ_{fish}$ while a composite sample of crayfish hepatopancreas (n=21) was determined to have a $TEQ_{fish}$ of 15.4 ng·kg$^{-1}$ (Dwernychuk et al. 1993). In Ontario, mussels (*Elliptio complenata*) sampled in 1986 from Frog Creek were found to contain less than the combined detection limit for all dioxin and furan congeners. $T_4CDD$ and $T_4CDF$ were found in a sample from Stanjikoming Bay (2.3 and 2.7 ng·kg$^{-1}$, respectively) downstream of a waste disposal site (Hayton et al. 1990).

There are only three studies that report PCDD/F concentrations in marine invertebrates (Table 24). A large study by the CPPA (1989) measured the levels of PCDD/Fs in marine invertebrates

at certain distances from numerous pulp mill outfalls along the Pacific coast in British Columbia. The $TEQ_{fish}$ (based on WHO 1998 TEFs) ranged from 0.2 to 72.36 $ng \cdot kg^{-1}$ in muscle tissues of shrimp, *Pandalus borealis*, prawn, and dungeness crab, *Cancer magister*. Higher TEQs were observed in hepatopancreas tissue (11.2 $ng \cdot kg^{-1}$ to 403.8 $ng \cdot kg^{-1}$) of dungeness crabs, *Cancer magister*, and the soft tissue of littleneck clams, *Protothaca staminea*, and oysters, *Crassostrea* spp., (0.42 $ng \cdot kg^{-1}$ to 101.91 $ng \cdot kg^{-1}$). Clements et al. (1987) examined the digestive glands of lobsters, *Homarus americanus*, captured in New Brunswick and Nova Scotia to determine their PCDD/F levels. $TEQ_{fish}$ ranged from 9.81 $ng \cdot kg^{-1}$ to 36.11 $ng \cdot kg^{-1}$. In Québec, Brochu et al. 1995 reported TEQs (based on I-TEFs) ranging from 0.23 to 2.99 $ng \cdot kg^{-1}$ in whelk and snow crab, *Chionoecetes opilio*, tissue, respectively.

### 6.5.2 *Freshwater Fish*

Several surveys of contaminants in the Fraser, Kootenay and Thompson Rivers in British Columbia were commissioned by Crestbrook Forest Industries Ltd. (CFI) (Dwernychuk et al. 1991a; 1991b; 1993; 1995; Table 25). These surveys were in response to a request by the British Columbia Ministry of the Environment in 1990, that the CFI initiate a baseline organochlorine monitoring program. These studies are an excellent basis for the comparison of temporal trends in contamination noted along the length of the Fraser and Kootenay Rivers. Both rivers are impacted by effluents from pulp and paper mills and other sources (e.g., sewage treatment plants). Fish species were sampled for PCDD/F levels in muscle and liver tissue and TEQs were calculated using WHO 1998 TEFs from congener specific information. It was found that TEQ concentrations decreased with increasing distance from the pulp and paper mills. In the Kootenay River, the liver tissue of the fish sampled contained the highest levels of PCDD/F. In 1990, the calculated $TEQ_{fish}$, $TEQ_{mammalian}$, and $TEQ_{avian}$ ranged from 0.9 – 32.5, 1.0 – 40.0, and 1.2 – 186.8 $ng \cdot kg^{-1}$, respectively, in the liver and muscle of fish sampled on the Kootenay River (Dwernychuk et al. 1991a). By 1996, liver and muscle tissues had levels of 0.6 – 4.6 $ng \cdot kg^{-1}$

($TEQ_{fish}$), 0.6 – 6.5 ng·kg$^{-1}$ ($TEQ_{mammalian}$), and 2.6 – 41.9 ng·kg$^{-1}$ ($TEQ_{avian}$) for similar fish species and similar sites along the Kootenay River (Dwernychuk et al. 1996). The Fraser River survey demonstrated a similar pattern of decreasing PCDD/F fish tissue levels. In 1990 the calculated $TEQ_{fish}$, $TEQ_{mammalian}$, and $TEQ_{avian}$ ranged from 1.5 – 113.3, 1.7 – 101.5, and 1.8 – 255.9 ng·kg$^{-1}$, respectively, in liver and muscle tissue (Dwernychuk et al. 1991b). A similar study along the length of the Fraser River was conducted in 1991 by McDonald et al. (1997). White sturgeon tissue samples were analyzed for PCDD/F concentrations. Red muscle and liver tissue contained the highest concentrations of PCDD/F (Table 25). The calculated $TEQ_{fish}$, $TEQ_{mammalian}$, and $TEQ_{avian}$ ranged from 0.31 – 80.8, 0.5 – 115.1, and 4.6 – 579.1 ng·kg$^{-1}$, respectively. In 1992, muscle and liver tissue in mountain whitefish (*Prosopium williamsoni*) were sampled at sites along the Fraser River (Dwernychuk et al. 1993). The calculated $TEQ_{fish}$, $TEQ_{mammalian}$, and $TEQ_{avian}$ ranged from 0.2 – 38.4, 0.3 – 39.5, and 0.3 – 46.8 ng·kg$^{-1}$, respectively (Table 25). The most recent survey of fish tissue levels in the Fraser River occurred in 1995 (Dwernychuk et al. 1995). The calculated $TEQ_{fish}$, $TEQ_{mammalian}$, and $TEQ_{avian}$ ranged from 0.3 – 21.6, 0.3 – 22.1, and 0.3 – 26.8 ng·kg$^{-1}$, respectively (Table 25). In each subsequent year of the survey of fish tissue concentrations in the Fraser and Kootenay Rivers, the maximum TEQ levels continued to decrease.

In the Great Lakes, fish tissue sampled in 1984 contained total PCDD/F ranging from 22 – 105.2 ng·kg$^{-1}$ in adult lake trout and walleye (*Stizostedion vitreum*) from five of the lakes (Table 25) (De Vault et al. 1989). Niimi (1996) examined several fish species for PCDD/F and found total PCDD/F ranged from 9.1 – 160 ng·kg$^{-1}$ in Lake Ontario.

### 6.5.3 *Marine Fish*

Very few studies examined the PCDD/F levels in marine fish (Table 26). Harfenist et al. (1995) sampled fish in the Fraser River estuary in 1991 in the vicinity of Westham and Iona Island,

British Columbia. Starry flounder (*Platichthys stellatus*), threespine stickleback (*Gasterosteus aculeatus*), peamouth chub (*Mylocheilus caurinus*), and Pacific staghorn sculpin (*Leptocottus armatus*) tissue was analyzed for PCDD/F concentrations. Only the concentrations of $T_4$CDD and $T_4$CDF were reported. Levels ranged from <2.1 – 17.2 ng·kg$^{-1}$.

### 6.5.4 *Reptiles*

Information on levels of PCDD/F in reptiles in Canada is limited to snapping turtles (*Chelydra serpentina*) (Table 27). Snapping turtles from the St. Lawrence River contained 41.7 – 355 ng·kg$^{-1}$ TEQ in liver tissue and 298.6 – 2094 ng·kg$^{-1}$ in fat tissue (based on TEFs from Safe 1990) (Ryan et al. 1986). Snapping turtle eggs from Lake Ontario contained from below detection up to 422.4 ng·kg$^{-1}$ $TEQ_{fish}$ (Bishop et al. 1996). Eggs taken from Algonquin Provincial Park had the lowest levels of PCDD/F reported. Bishop et al. (1994) determined ecological or physiological parameters such as individual variation in feeding locations and/or food preferences and metabolism may be more important in determining contaminant levels than age, clutch size, or mass.

### 6.5.5 *Birds*

There is a great deal of information available on the levels of PCDD/F found in bird tissues (Table 28). This body of data is largely due to the use of bird tissues, in particular eggs, to monitor the concentrations of organochlorine contaminants in the environment (Ewins et al. 1994). Population declines, physiological abnormalities, and egg shell thinning have been indicators of potential organochlorine exposure in birds, particularly among piscivorous birds (Ewins et al. 1994).

The Great Lakes were known to be among the most heavily contaminated freshwater ecosystems in the 1960s and 1970s. For example, $T_4CDD$ and $T_4CDF$ levels as high 2350 $ng \cdot kg^{-1}$ were found herring gull eggs collected from 1971-82. The introduction of regulatory controls has helped reduce contaminant levels in Great Lakes fish and subsequently contaminants in aquatic birds (Ewins et al. 1994). A more recent study by Jones et al. (1994) conducted in 1989 on herring gulls (*Larus argentatus*) found TEQ levels (based on H4IIE bioassay) ranging from 96.9 – 399 $ng \cdot kg^{-1}$. Double–crested cormorants from the same region had TEQs ranging from 17.9 – 382.3 $ng \cdot kg^{-1}$.

In other areas, a temporal trend in PCDD/F levels in bird tissues is not immediately apparent in the data (Table 28). This reflects the differences in the feeding behaviour, and metabolism of the birds studied, prey items consumed, and their exposure to PCDD/F sources and emissions. In British Columbia, various species of birds were analyzed for PCDD/F congeners in several studies over a similar time period at numerous points (Elliott et al. 1996c; 1996d; Elliott & Martin 1998; Vermeer et al. 1993). Levels of $T_4CDD$ and $T_4CDF$ ranged from <DL – 3560 $ng \cdot kg^{-1}$ in bird tissue. The highest concentrations were detected in lipid rich tissue (e.g., liver and eggs) as expected due to the hydrophobic nature of dioxins and furans. In a study of the Peace and Athabasca Rivers in northern Alberta, four of six canvasbacks (livers contained from <0.2 to 1.4 $ng \cdot kg^{-1}$ of 1,2,3,7,8-PCDD, $T_4CDF$, and 2,3,4,7,8-PCDF with $T_4CDF$ being the most commonly detected (Wayland et al. 1995b).

### 6.5.6 *Terrestrial Mammals*

There is a paucity of data available on PCDD/PCDF levels in terrestrial mammals in Canada (Table 29). Hebert et al. (1996) found very low PCDD/F levels in caribou (*Rangifer tarandus*) fat and muscle. Mink from La Tuque, Québec had $TEQ_{mammalian}$ of 2.3 – 32.5 $ng \cdot kg^{-1}$ (Champoux 1986). In a study conducted in the Northwest Territories, essentially no PCDD/Fs were found in

mink liver (<1 ng·kg$^{-1}$) (Muir et al. 1996a). In mink form the Peace and Athabasca Rivers in northern Alberta, only TCDD was detected in two of the three pooled liver samples at 0.2 and 0.6 ng·kg$^{-1}$ (Wayland 1995a).

### 6.5.7 *Marine Mammals*

There is a large amount of information available on the concentrations of PCDD/F in marine mammals in Canada (Table 14). Marine mammals tend to be long lived and are at the top of the food chain. They tend to accumulate lipophilic substances like PCDD/F in tissues with high lipid content (Muir et al. 1996). Off the Pacific coast of Canada, relatively low levels of $T_4$CDD and $T_4$CDF were reported in studies on porpoise and whale species (Burlinson 1991; Jarman et al. 1996). $T_4$CDD concentrations ranged from below detection to 3.3 ng·kg$^{-1}$ and $T_4$CDF concentrations ranged from <2 to 109 ng·kg$^{-1}$. In harbour seal (*Phoca vitulina*) tissue $T_4$CDD concentrations ranged from below detection to 6.9 ng·kg$^{-1}$ and $T_4$CDF concentrations ranged from 3.7 to 62 ng·kg$^{-1}$ (Addison et al. 1996). In the Arctic, marine mammals including polar bear (*Thalarctos maritimus*), beluga (*Delphinapterus leucas*), narwhal (*Monodon monoceros*) and ringed seal (*Phoca hispida*) were reported to have $T_4$CDD concentrations ranging from <2 to 37 ng·kg$^{-1}$, $T_4$CDF concentrations ranging from 3.7 to 61.8 ng·kg$^{-1}$, 1,2,3,6,7,8-HCDD ranging from <4 to 9 ng·kg$^{-1}$, and OCDD ranging from <8 to 44 ng·kg$^{-1}$ (Norstrom et al. 1990). In a study of PCDD/F concentrations in beluga whale for the St. Lawrence estuary, no PCDD/F were detected in liver (Muir et al. 1996b). Low levels of PCDF were detected in the beluga blubber samples while PCDDs were undetectable; the highest concentration of PCDF (8 ng·kg$^{-1}$) were detected in blubber of a male (Muir et al. 1996b). There is a lack of data from marine mammals on the Atlantic coast. Muir and Norstrom (1990) reported very low levels (below detection) of PCDD/F in white-beaked dolphins, beluga and pilot whale. The variability among marine mammal species may reflect differences in exposure related to trophic status and proximity to PCDD/F sources, sex of animals sampled and life cycle duration.

# 7. OVERVIEW OF TOXICITY

A multitude of toxic responses to PCDD/F exposure have been described in the scientific literature. The aim of the present document is not to conduct an exhaustive review of the effects of PCDD/F exposure, but rather to scrutinize the data relevant to the derivation of Canadian EQGs and to provide sufficient background information to orient the reader in this context. Several recent reviews on the affects of PCDD/Fs on aquatic organisms, (Fletcher and McKay 1993; Walker and Peterson 1994a; Fitzsimons 1995; Grimwood and Dobbs 1995), birds (Bosveld and van den Berg 1994; Henshel 1998), mammals (Neubert 1992; Sauer et al. 1994; Birnbaum 1995a, b; Kerkvliet 1995) and biota in general (Peterson et al. 1993; Vanden Heuvel and Lucier 1993) are available and the reader is directed to these reviews for more detailed information on topics that are summarized in this document. Responses that have been commonly documented include mortality (often delayed), decreased body weight gain, decreased feed consumption, thymic atrophy, fin necrosis, histopathologic effects, immunotoxicity, developmental and reproductive effects, biochemical effects, neurotoxicity, and carcinogenesis. PCDD/Fs are also known to disrupt the endocrine system which could have serious repercussions on sexual development. Clearly, it is unlikely that the complete spectrum of effects would be observed in any single species but the data indicate that PCDD/Fs and related compounds elicit the same qualitative pattern of responses within each species (Safe 1986). Effects observed vary with a number of factors including the dose of the toxic substance, the congener tested, and life stage, strain, species, and gender of the organisms tested.

## 7.1 Mode of Action

### 7.1.1 *Ah Receptor Binding*

PCDD/Fs are thought to elicit most, if not all, of their toxicity via the aryl hydrocarbon (*Ah*) receptor, a protein conserved across mammals, birds, and fish (Clark et al. 1992; Vanden Heuvel and Lucier 1993). Dioxin binding to the *Ah* receptor was first hypothesised by Poland and Glover (1973), based on experiments that quantified aryl hydrocarbon hydroxylase (AHH) activity in chicken eggs following PCDD injection. It is now known that the *Ah* receptor is a member of the basic helix-loop-helix family of proteins. As with the steroid receptors, the *Ah* receptor exists in a multiprotein complex in association with heat shock and other proteins. Upon ligand binding in the cytosol, a conformational change in the receptor results in the release of the proteins (Birnbaum 1995b). The ligand-receptor complex is transported to the cell nucleus where it binds to DNA at specific sequences and modifies gene transcription (Peterson et al. 1993). It has been postulated that because the *Ah* locus controls the expression of not only AHH, but several other genes as well, the toxicity of dioxin-like compounds may result from the expression or repression of multiple genes (Bryan et al. 1987). Binding of dioxin-like compounds to the *Ah* receptor correlates well to the induction of mixed-function oxygenase (MFO) enzyme systems such as cytochrome P-450-1A1 isozyme (CYP1A1) as measured by ethoxyresorufin *O*-deethylase (EROD) and cytochrome P-450-1A2 (CYP1A2) as measured by acetanilide-4-hydroxylation (Safe 1990; Brouwer 1991; De Vito et al. 1993). These enzymes belong to a family of 12 cytochrome P-450 isozymes (i.e., a group of enzymes which are chemically distinct but functionally alike, also referred to as MFO systems) that are found in liver endoplasmic reticulum and that may be involved in biotransformation, conjugation and removal, or bioactivation of certain lipophilic foreign compounds. Mammals and birds generally have two cytochromes in the P-450-1A subfamily, known as 1A1 and 1A2, while only one version of cytochrome P-450-1A has been found in fish (Stegeman and Hahn 1994).

The role of the *Ah* receptor protein in the mechanism of action of toxic halogenated aryl hydrocarbons has been thoroughly investigated and satisfies most of the specific criteria that support a receptor-mediated cellular process (Safe 1986). These criteria include: i) the existence of a finite number of specific binding or receptor sites and therefore saturable binding; ii) high

affinity ligand binding that is commensurate with the usually low levels of circulating hormones; iii) stereoselective binding capacity for the receptor; iv) tissue or organ response specificity for the receptor ligand; and v) a correlation between binding affinities, receptor occupancy, and the magnitude of the response.

Evidence of the existence of the *Ah* receptor protein and/or P-450-1A1 inducibility has been found in all vertebrates (mammalian, avian, and fish species) with the notable exception of Atlantic hagfish (*Myxine glutinosa*) and sea lamprey (*Petromyzon marinus)*, both of which are species that belong to the agnathans, a primitive class of jawless aquatic vertebrates (Hahn et al. 1992; Vanden Heuvel and Lucier 1993). Conservation of the *Ah* receptor across species lends support to the common mode of action theory. Further, that the *Ah* receptor protein has not been detected in invertebrates or in plants may explain why the toxic effects of $T_4$CDD do not appear to be conferred upon these taxonomic groups.

### 7.1.2 *Receptor-Occupancy and Toxic Threshold Theories*

Supporters of the receptor-occupancy theory argue that a certain number of the receptors must be occupied before any biological response is expressed. While there is consensus that some *Ah* receptors must be occupied, the exact number is a matter of considerable controversy. The presence/absence of the *Ah* receptor alone does not explain observed differences in species and tissue effects from PCDD/Fs (Vanden Heuvel and Lucier 1993). While hepatic *Ah* receptor levels and binding affinity for [$^3$H] 2,3,7,8-$T_4$CDD in several species including pigs, rats, hamsters and nonhuman primates, are similar, there are distinct differences in maximal P-450-1A1 induction and toxicity (Safe 1986). For example, although the guinea pig and the hamster have comparable *Ah* receptor characteristics, the acute lethality and maximal P-450-1A1 induction by $T_4$CDD varies over a 5000-fold range between the two rodents (Vickers et al. 1985). Furthermore, there is little difference in the concentration of the *Ah* receptor in various rodent

tissues (Denison et al. 1986). Therefore, although the presence of the *Ah* receptor is required for key biochemical and biological responses to PCDD/Fs, its existence does not fully explain the qualitative and quantitative differences in biological responses.

The key point of the receptor-occupancy theory is that there is a dose below which the receptor does not function and, if it is not activated, there can be no effect. This implies that there is a "safe" or practical "threshold" below which no toxic effects occur. No toxic effects are known to occur at levels below those required for enzyme induction (Roberts 1991). Increased activity (induction relative to 'normal' or control levels) of these enzymes (i.e., EROD, AHH) is often used as a surrogate measure for receptor binding, and hence, contaminant exposure. Thus, induction of hepatic microsomal enzymes may be one of the earliest and most sensitive indicators of biological response to PCDD/F exposure. While it may be argued that toxic effects from PCDD/F exposure will not occur without enzyme induction (i.e., the receptor must be activated), to date there is no clear evidence to suggest that if enzyme induction has occurred, a toxic response will necessarily follow. Enzyme induction is a biochemical response to PCDD/F exposure, but enzyme activity at or above the threshold (induction, relative to control levels) does not guarantee that a toxic response is imminent.

From a research standpoint and also in terms of risk assessment, the threshold concept has been questioned. Tritscher et al. (1992) investigated the dose-response relationships for induction of cytochrome P-450-1A1 and 1A2 following chronic exposure of diethylnitrosamine-initiated and noninitiated female Sprague-Dawley rats to $T_4CDD$ in a liver tumour promotion model. In this study, $T_4CDD$ was administered biweekly by gavage at doses equivalent to 3.5, 10.7, 35.7, and 125 $ng \cdot kg^{-1}$ $bw \cdot d^{-1}$ for 30 weeks. Mathematical analysis of isozyme induction data as a function of liver $T_4CDD$ concentrations indicated that the best fit for these data are inconsistent with a threshold above zero for the induction response. The best fitting curve was linear for induction of both P-450-1A1 and 1A2 at low $T_4CDD$ concentrations. The authors concluded that, in a chronic exposure experiment, their data suggest that there is no evidence of a threshold for

hepatic P-450-1A1 and 1A2. It is possible, however, that the relationship may take on a sigmoidal curve below the lowest $T_4CDD$ liver concentration (480 ± 190 $ng \cdot kg^{-1}$ ww) measured. Therefore, this study does not conclusively rule out the existence of a threshold. In another publication based on the same rat tumour promotion model, it was argue that a risk assessment approach involving the use of P-450-1A1 induction as a surrogate marker for cancer (and application of an uncertainty factor) may be flawed (Clark et al. 1992). It was shown that the dose-response relationships for $T_4CDD$-mediated increases in hepatic cell proliferation and preneoplastic lesions were distinctly different from those depicting P-450-1A1 induction in that much higher doses of $T_4CDD$ were required to detect increases in cell proliferation than those needed to produce enzyme induction. The contention then is that the threshold for enzyme induction is not appropriate as a basis for assessing risk because in this particular case, it was shown to be too conservative with respect to a demonstrated toxic effect (cancer promotion).

This rationalization is the basis for the opposing perspective that because the enzyme induction threshold is conservative it may be the *most* appropriate measure to use in risk assessment. In a sense, induction of P-450-1A1 enzymes is the organism's first response (or attempt at defence) to PCDD/F exposure. While this biochemical response does not mean that toxic responses will necessarily occur; to date, it is the most sensitive marker indicating the risk of an adverse biological effect or toxic response.

### 7.1.3 *Binding Affinity*

While the qualitative and quantitative differences in biological responses to PCDD/F exposure cannot be related to differences in *Ah* receptor levels, differences in biological responses to the various PCDD/F congeners are related to binding affinity to the *Ah* receptor. The receptor binding affinities and toxicity of halogenated aryl hydrocarbons are dependent on their molecular structure (i.e., degree of chlorination and substitution pattern). According to Safe (1987),

interactions with the *Ah* receptor are highly stereoselective and this accounts for the marked effects of molecular structure on the biologic and toxic potencies of PCDD/Fs. Chlorine substitution in the lateral positions (2,3,7,8) strongly affects the interaction between the PCDD ligands and the cytosolic receptor protein (Safe 1986). $T_4$CDD has the greatest binding affinity for the *Ah* receptor of any known substance and it is also the congener that has been shown to exert the most potent toxic effects; $T_4$CDD is regarded as one of the most toxic manmade (xenobiotic) compounds known. Other toxic PCDD/F congeners are approximate isomers of $T_4$CDD and are substituted in at least three of the four lateral 2,3,7, and 8 positions (Safe et al. 1989). With increasing Cl substitution at the nonlateral positions 1,4,6, and 9, there is a marked decrease in receptor binding affinity, and hence biological potency and toxicity. According to Safe (1986), the stepwise addition of Cl at these nonlateral positions would result in several structural changes in the more highly chlorinated PCDD/Fs including increased molecular size and volume, increased lipophilicity, a possible decrease in PCDD/Fs coplanarity associated with steric crowding, and decreased aromatic ring electron density (due to the additional electronegative Cl groups) all of which render them less toxic. The axiom that binding affinity for the *Ah* receptor, as influenced by molecular structure, is primarily responsible for the degree of the adverse or toxic responses exerted is further substantiated by the empirical finding that the order of toxic potency among PCDD/F congeners is generally maintained across test organisms from different taxonomic groups (i.e., 2,3,7,8-TCDD ≥ 1,2,3,7,8-PCDD ≥ 2,3,7,8-TCDF ≥ 1,2,3,7,8-PCDF etc.) (Mason et al. 1986).

## 7.2 Biotransformation

Biotransformation refers to the process in which one or more biochemical reactions transform a parent compound into a derivative that 1) may be used satisfy metabolic requirements, or 2) is detoxified and/or excretable or non-absorable (Norstrom and Letcher 1997). Biotransformation of xenobiotics by cytochrome P-450 is not always beneficial because there are many cases where

the metabolites are more toxic or biologically active than the parent compound (i.e., PAH compounds such as benzo[a]pyrene; McFarland and Clarke 1989). Limited data suggest that PCDD/F metabolites are much less toxic than their parent hydrocarbons (Weber et al. 1982). Moreover, metabolites of PCDD/Fs are several orders of magnitude less active in terms of receptor-mediated biochemical and toxicological effects in the rat (Mason and Safe 1986). The metabolism of $T_4$CDD and related compounds (with the exception of OCDD) is necessary prior to urinary and biliary elimination; therefore, the rate of excretion of these compounds is dependent on the rate of transformation (van den Berg et al. 1994). The biotransformation of PCDD/F congeners has been studied in several species. Metabolites of $T_4$CDD in rat livers (*in vivo*) included dihydroxy-$T_3$CDD, dihydroxy-tetrachlorodiphenyl ether, and 2-hydroxy-1,3,7,8-TCDD (Poiger and Buser 1984) while primary rat hepatocyte cultures (*in vitro*) transformed the parent compound into 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TCDD (Sawahata et al. 1982). Poiger and Buser (1984) found that the major metabolite of $T_4$CDD in the dog was 2-hydroxy-1,3,7,8-TCDD. Gobas and Schrap (1990) evaluated the ability of guppies to metabolize dioxins and found mono-, di- and tri-hydroxy transformation products. In a review of the impact of PCDD/Fs on human and environmental health, Ahlborg et al. (1992) concluded that the adverse effects from PCDD/Fs are primarily caused by the parent compounds, rather than by metabolites.

## 7.3 Toxic Equivalency Factors (TEFs) and Toxic Equivalents (TEQs) - Toxicity

As explained in Section 2.2, TEFs may be used to express (or 'convert') concentrations of compounds that exert dioxin-like effects to 'equivalent' values as if they were $T_4$CDD. In other words, TEQs allow risk assessors to compare quantitatively the toxic potency of compounds that are structurally similar (i.e. 2,3,7,8-substituted PCDDs and PCDFs, non-ortho and mono-ortho-substituted coplanar PCBs) to the congener that is thought to be the most toxic environmental contaminant, $T_4$CDD. The rationale behind the TEF/TEQ approach is that compounds that share

similar molecular structures to $T_4CDD$ exhibit similar toxicities but differ in their potency. This empirical finding may be explained by the fact that dioxin-like toxicity is initiated by binding of a compound to the *Ah* receptor and that this binding requires certain molecular structural characteristics which are shared by $T_4CDD$ and compounds that are isosteric to it (McFarland et al. 1993). Subsequently, a compound may be assigned a TEF that expresses its toxicity as a fraction of $T_4CDD$ toxicity. Summation of the individual products of TEFs and compound concentrations in a sample yields a TEQ. For the purposes of risk assessment, the TEQ may then be used as if it were the concentration of $T_4CDD$ in the sample. TEFs and TEQs are valuable tools because they provide the common 'currency' (units) via which sensitive or toxic endpoints brought about by different PCDD/F congeners may be compared. Moreover, the TEF/TEQ approach facilitates the assessment of the potency of complex environmental mixtures which may contain a wide array of substances that contribute to the 'total' toxic effect.

The TEF/TEQ approach relies on the availability of appropriate TEFs and assumes that the individual compounds act via a common mechanism and that their toxic effects are cumulative (additive). Therefore, analytically-derived TEQs necessarily ignore potential synergistic or antagonistic effects amongst the individual compounds in a mixture. In general, it appears that toxic effects are additive but some evidence suggests that modulation of toxic effects may occur and that some compounds in mixtures do not produce strictly additive effects.

Evidence for near-additivity was found using early life stage (ELS) tests with rainbow and lake trout (Walker et al. 1996). Eleven $T_4CDD$-like congeners (including four PCDDs, four PCDFs and three coplanar PCBs) and three non-$T_4CDD$ like congeners (i.e. two mono-ortho and one di-ortho substituted PCBs) were combined at ratios typically found in Lake Michigan trout. The potency of the mixture, expressed as a total TEQ (TEFs from Walker and Peterson 1991) was slightly less than additive compared to $T_4CDD$ alone for producing early life stage mortality; the dose-response curves were parallel for mortality versus egg TEQ or egg $T_4CDD$, but that for egg TEQ was shifted to the right of the $T_4CDD$ curve by 1.3 to 1.8 fold, as indicated by the $LD_{50}$

values (Walker et al. 1996). These researchers pointed out that although the congeners tested did not produce strictly additive responses, the deviation (1.3 to 1.8 fold) was much less than the commonly applied uncertainty factor of 10 used in ecological risk assessments, and therefore, strict additivity under certain assessments may not critically alter the conclusions reached in the assessment.

Hornung et al. (1996a, b) also used early life stage mortality in rainbow trout to assess how pairs of PBDDs (polybrominated dibenzo-*p*-dioxins), PBDFs (polybrominated dibenzofurans) and PBBs (polybrominated biphenyls) congeners interact to produce $T_4$CDD-like toxicity. Fertilized rainbow trout eggs were injected with graded doses of each congener alone or fixed ratios of paired congeners including 2,3,7,8-TBDD/1,2,3,7,8-PBDD, 2,3,7,8-TBDD/1,2,3,7,8-PBDF, 1,2,3,7,8-PBDD/2,3,4,7,8-PBDF, and 2,3,4,7,8-PBDF/3,3',4,4'-TBB. Interactions between congener pairs were additive in all cases in terms of producing sac fry mortality relative to responses evoked by single congeners (Hornung et al. 1996a, b). Similarly, Bol et al. (1989) found that TEQs derived using early life stage mortality of rainbow trout as an endpoint corresponded with analytical TEQs for isotoxic mixtures of $T_4$CDD with 1,2,3,7,8-PCDD, 2,3,4,7,8-PCDF, and 1,2,3,7,8,9-HCDF.

The additivity of among PCDD/F congeners has also been reported in mammalian organisms. For example, in a study in which pregnant mice were dosed $T_4$CDD, $T_4$CDF, or a combination of both, it was concluded that for cleft palate frequency in fetuses, $T_4$CDD/F toxicity is additive, with one unit of $T_4$CDD approximately equal to 30 units of $T_4$CDF (Weber et al. 1985). Whyte et al. (1998) found that *Ah* receptor compounds extracted from lake trout livers acted in an additive fashion in both mammalian and piscine systems. Other results also support the additive concept for 2,3,7,8-substituted PCDDs and PCDFs congeners (Birnbaum et al. 1987; Pleuss et al. 1988). In contrast to strict additivity, however, Bol et al. (1989) also noted that mixtures of $T_4$CDD or $T_4$CDF with other cytochrome P-450 inducers were more than additive. For example, two PCBs (3,4,3',4'-TCB and 2,4,5,2',4',5'-HCB), and 1,3,6,8-TCDF had more than additive

effects. Synergism was also noted between 3,4,3',4'-TCB and 2,3,4,7,8-PCDF. The authors noted that the more than additive effects were dependent upon water temperature and concluded that the most logical explanation for these synergistic effects was a mechanism of action regulated by more than one receptor. Vickers et al. (1985) also suggested the possibility of more than one receptor, each producing different effects.

Conflicting evidence regarding the strict additivity concept and the use of TEFs/TEQs has been reported. To address the assumption that all interactions of PCDDs are additive, acute toxicity studies with four different PCDDs (i.e., $T_4$CDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HCDD and 1,2,3,4,6,7,8-HCDD) were conducted and the $LD_{20}$, $LD_{50}$, and $LD_{80}$ for each congener in male Sprague Dawley rats were determined (Stahl et al. 1992). Equipotent doses, assuming additive toxicity, of a mixture containing all four congeners were then prepared using this information. The authors concluded that the dose-response (mortality, body weight change) of the rats to the mixture confirmed the hypothesis of strict additivity in the acute toxicity of the four congeners. Several years later, the same group of researchers measured the actual concentrations of the four congeners in the rats' livers and came to a different conclusion. Rozman et al. (1995) found that the absorption of the four PCDDs by rats after oral administration decreased in the order of 2,3,7,8-TCDD ≥ 1,2,3,7,8-PCDD >1,2,3,4,7,8-HCDD > 1,2,3,4,6,7,8-HCDD, indicating that the dose was an incomplete surrogate of exposure in the earlier investigation. These findings suggested that the relative potency of the higher chlorinated congeners was slightly greater by approximately a factor of two, than suggested in the earlier publication (Stahl et al. 1992), because of the reduced absorption, while the contribution to total potency of the lower chlorinated congeners was slightly higher by approximately a factor of two due to increased relative liver concentrations (Rozman et al. 1995).

Other less-than additive effects of PCDD/F mixtures have also been demonstrated. For example, 1,3,6,8-TCDF was reported to have had an antagonistic effect on the enzyme induction caused by $T_4$CDD *in vivo* in C57BL/6J mice (Bannister and Safe 1987) as well as in rat hepatoma cells in

culture (Keys et al. 1986). Moreover, Nagao et al (1993) studied the potency of $T_4$CDD, 2,3,4,7,8-PCDF, and of one PCDD and two PCDF (defined) mixtures in random-bred albino mice and found that the dose-response curve for 2,3,4,7,8-PCDF was similar to that for $T_4$CDD. Application of the I-TEF for 2,3,4,7,8-PCDF of 0.5, however, overestimated this congeners teratogenic potency of by approximately 2.5 fold under their experimental conditions. Assessment of the cleft palate frequency on the basis of calculated TEQs (using I-TEFs) showed that the potencies of the two PCDF mixtures studied were also overestimated while the cleft palate inducing potency for the PCDD mixture largely agreed with the predicted outcomes. These authors concluded that the use of I-TEFs are conservative when attempting to assess the cleft palate incidence induced by PCDF mixtures in mice. The results of this study indicate that the individual congeners in the PCDF mixtures may have interacted in an antagonistic manner, or that the TEFs used may have been inappropriate for this specific experimental system. I-TEFs were developed from short-term whole animal studies (such as reproductive effects), subchronic effects data (such as thymic atrophy, reduced body weight gain, etc.) and acute toxicity data (lethality) for mammalian systems because data from long-term whole animal studies involving carcinogenicity were not available at the time (Kutz et al. 1990). It is possible that I-TEFs, although they were developed from data pertaining to mammals, may not have been appropriate for the teratogenic endpoints used in this study.

In recent years, the H4IIE rat hepatoma cell bioassay has been developed specifically to measure the net effect of all *Ah* receptor active compounds in complex and highly varied environmental mixtures (Bryan et al. 1987; Tillitt et al. 1991; Ankely et al. 1993; Ludwig et al. 1996). The H4IIE bioassay measures the integrated potency of sample extracts, often containing complex mixtures of dioxin-like compounds, to induce specific cytochrome P-450-requiring MFO enzymes in isolated rat hepatoma cells (Giesy et al. 1994). Briefly, cells are exposed to sample extracts (typically containing mixtures of compounds) and subsequent enzyme induction (e.g., EROD) is measured and compared to induction measured in cells exposed to known concentrations of $T_4$CDD. Results are thus reported as equivalents in toxic potency relative to

$T_4CDD$. Similar bioassays have been developed using primary chicken embryo hepatocyte (CEH) cultures and rainbow trout hepatoma cell cultures [remodulated lightning trout (RLT) bioassay] to measure TEQs that are relevant to birds and fish, respectively (Kennedy et al. 1996a; Richter et al. 1997). Although the bioassay method is not dependent on strict additivity, it provides no qualitative information about the identity of substances that exist in the sample, except that the substances are capable of inducing an *Ah* receptor response. But, when used concurrently with analytical instrumentation, bioassay techniques can determine whether all of the TEQs in an extract have been accounted for and whether there are non-additive interactions among congeners. For example, bioassay derived TEQs measured with H4IIE or RLT cell lines for livers of lake trout collected from Lakes Superior and Ontario did not differ significantly from analytically derived TEQs, suggesting that the *Ah* receptor active congeners in the sample extract were accounted for by congener analysis and that they acted in an additive fashion (Whyte et al. 1998).

# 8. TOXICITY TO AQUATIC ORGANISMS

Acute and chronic PCDD/F toxicity data, including lethal, growth, and reproductive endpoints, for amphibians, fish, invertebrates, and plants are summarized in Table 31. Where available, data entries include information on water exposure concentrations and/or dosage rates, nominal tissue concentrations, and measured tissue concentrations, with PCDD/F levels expressed on a TEQ basis using appropriate fish TEFs (van den Berg et al. 1998). Furthermore, NOELs and LOELs are indicated wherever possible and statistical significance of endpoints was included if it was reported by the authors.

## 8.1 Effects From PCDD/Fs in Freshwater

### 8.1.1 *Fish*

Represented in Table 31 are 9 families and 19 species of fish, of which 7 families and 15 species are resident in Canadian waters. Together these data indicate that freshwater fish exhibit a wide range of sensitivities to PCDD/Fs. Exposure to $T_4$CDD and related compounds may result in a variety of adverse effects in fish, including: reduced survival and growth rates (Mehrle et al. 1988), fin necrosis (Kleeman et al. 1988), edema (Helder 1981), reproductive failure (Walker et al. 1991, 1992), and teratogenic effects (Helder 1981).

Comparison of the LOELs (Table 31) for a variety of endpoints (i.e., sensitive and toxic) indicates that rainbow trout tests yielded the most sensitive response both on a water concentration exposure basis and nominal tissue dose/measured tissue residue basis. Mehrle et al. (1988) found that the LOEL for mortality in juvenile (0.38 g) rainbow trout exposed to graded concentrations of $T_4$CDD (0.001, 0.038, 0.079 and 0.176 $ng \cdot L^{-1}$) for 28 d and subsequently maintained in clean water for another 28 d, was 0.038 $ng \cdot L^{-1}$. This LOEL also applied to

reductions in growth after 28 d of exposure to the graded levels of $T_4CDD$ in water. Similarly, Miller et al. (1973) exposed juvenile (3.5 g) coho salmon *(Oncorhychus kisutch)* to nominal concentrations of $T_4CDD$ ranging from 0 to 5.6 $ng \cdot L^{-1}$. Using pooled data for 24, 48, and 96 h exposures, concentrations as low as 0.056 $ng \cdot L^{-1}$ were coincident with increased mortality measured within 60 d following exposure. The statistical significance of the difference in survival between treatment and control groups was not reported. In a subsequent publication based on the same data, significant mortality of *O. kisutch* fry was observed only in the treatment groups exposed to 5.6 $ng \cdot L^{-1}$ or more for 48 and 96 hours (Miller et al. 1979). Lake trout and brook trout exhibited the least sensitivity of the salmonids, when exposed as eggs to waterborne $T_4CDD$, with LOELs of 10 $ng \cdot L^{-1}$ for mortality to swim-up (Spitsbergen et al. 1991) and 8 $ng \cdot L^{-1}$ for sac fry mortality (Walker and Peterson 1994b), respectively. While embryotoxicity assays for sensitive endpoints using fish from non-salmonid taxonomic groups yielded relatively low LOELs (fathead minnow, $LOEL_{lesions}$ = 0.37 $ng \cdot L^{-1}$, Olivieri and Cooper 1997; medaka, $LOEL_{lesions}$ = 0.4 $ng \cdot L^{-1}$, Wisk and Cooper 1990a, b; northern pike, *Esox lucius*, $LOEL_{reduced\ growth}$ = 0.1 $ng \cdot L^{-1}$; guppy, $LOEL_{fin\ necrosis}$ = 0.1 $ng \cdot L^{-1}$; Miller et al. 1979), these parameters are still an order of magnitude higher than the mortality and growth response LOEL of 0.038 $ng \cdot L^{-1}$ for rainbow trout (Mehrle et al. 1988).

As mentioned earlier, the most sensitive response (lowest LOEL) to PCDD/Fs from the available data, based on nominal tissue dose/measured tissue residues, were elicited from rainbow trout. In addition to evaluating the effects of graded water concentrations of $T_4CDD$ on juvenile rainbow trout, Mehrle et al. (1988) also examined their response to graded water concentrations of $T_4CDF$ (<0.001, 0.02, 0.05, 0.09, 0.20 and 0.44 ng $TEQ \cdot L^{-1}$). Following a 28 day depuration phase that was preceded by a 28 day exposure phase, the LOEL for mortality was 0.027 µg $TEQ \cdot kg^{-1}$ ww. Similar LOELs, ranging from 0.036 to 0.061 µg $TEQ \cdot kg^{-1}$ ww (in eggs), for lake trout sac fry mortality have also been reported (Guiney et al. 1996). Under a constant (8°C) or variable water temperature regime (8 to 3 to 8°C) for each test group, these researchers exposed eggs taken from western (Fifty Point) and eastern (Stony Island) Lake Ontario and

hatchery lake trout to 0-150 ng $T_4CDD \cdot L^{-1}$ and eggs from southwestern Lake Superior (Gull Island) lake trout to 0-100 ng $T_4CDD \cdot L^{-1}$. Eggs exposed to $T_4CDD$ and maintained at either 8°C or 8-3-8°C had similar sac fry moralities associated with blue sac disease (Guiney et al. 1996). While salmonids appear to be the most sensitive taxonomic group, the $LOEL_{lesions}$ (0.04 µg $TEQ \cdot kg^{-1}$ ww) for fathead minnows exposed as eggs to 0 to 10.2 ng $T_4CDD \cdot L^{-1}$ (Olivieri and Cooper 1997) falls within the range of LOELs determined for lake trout. Similarly, the mean nominal tissue dose $LOEL_{mortality}$ for coho salmon (3.5 g) exposed for 24, 48, and 96 h to waterborne $T_4CDD$ by Miller et al. (1973) was 0.056 µg $TEQ \cdot kg^{-1}$ ww, but the statistical significance of this finding was not reported and the nominal tissue dose was calculated under the assumption that the fish would take up all of the available $T_4CDD$ (i.e., no loss of $T_4CDD$ due to volatilization, binding to surfaces of the holding container etc.). Other salmonids, namely brook trout and cisco/lake herring (*Coregonus artedii*) are less sensitive than rainbow trout or lake trout to the effects of PCDD/Fs by at least an order of magnitude on a tissue residue basis. Walker and Peterson (1994b) found that the $LOEL_{mortality}$ for brook trout sac fry exposed as eggs to 0, 4, 6, 8, 10, 15, 20 or 30 ng $T_4CDD \cdot L^{-1}$ was 0.185 µg $\cdot kg^{-1}$ ww egg, while cisco/lake herring eggs exposed for 20 minutes to 31 ng $T_4CDD \cdot L^{-1}$ displayed a LOEL for survival of 0.270 µg $\cdot kg^{-1}$ ww egg (Elonen et al. 1998). Similarly, medaka exposed to 0, 0.5, 2.4, 7.0, 12.0, 33.5, or 57.9 ng $T_4CDD \cdot L^{-1}$ as eggs had a $LOEL_{lesions}$ of 0.3 µg $\cdot kg^{-1}$ ww dechlorinated embryo. Other taxonomic groups for which data are available reveal less sensitive responses. Elonen et al. (1998) exposed eggs from northern pike, channel catfish (*Ictalurus punctatus*) and white sucker (*Catastomus commersoni*) for various times to waterborne $T_4CDD$ (208, 31, and 285 ng $T_4CDD \cdot L^{-1}$, respectively) and determined that the LOELs for survival were 1.800, 0.855, and 1.960 µg $\cdot kg^{-1}$ ww egg, respectively.

Biochemical responses of fish exposed to PCDD/Fs (e.g., enzyme induction) are discussed in detail in a separate section below (see Section 8.3).

### 8.1.2 *Amphibians and Reptiles*

Few researchers have addressed the responses of amphibians to PCDD/Fs but limited data indicate that this group of organisms is relatively insensitive to the toxic effects of $T_4CDD$. Single doses of $T_4CDD$, administered by interperitoneal (ip) injection, of up to 1 000 $\mu g \cdot kg^{-1}$ body weight (bw) to bull frog (*Rana catesbeiana*) tadpoles and 500 $\mu g \cdot kg^{-1}$ bw to adults, for a period of 50 and 35 d , respectively, failed to produce any dose related effects on survival (Beatty et al. 1976). American toad (*Bufo americanus*) and green frog (*R. clamitans*) eggs exposed for 24-hr to 0.003-30 and 0.3-100 $\mu g$ $T_4CDD \cdot L^{-1}$, respectively, showed no significant increase in mortality relative to controls (Jung and Walker 1997). Mean concentrations as high as 19 331 and 73 717 ng $T_4CDD \cdot kg^{-1}$ were measured in the American toad and green frog eggs, respectively. Leopard frogs (*R. pipiens*) are more sensitive to the toxic effects of $T_4CDD$ as mortality was significantly increased (>10%) in eggs that had been exposed 3 $\mu g \cdot L^{-1}$ and displayed a mean concentration of 17 486 $ng \cdot kg^{-1}$ (Jung and Walker 1997). No information was found on the toxic effects of PCDD/Fs to reptiles.

### 8.1.3 *Invertebrates*

In general, invertebrates appear to be less sensitive to the toxic effects of water-borne $T_4CDD$ than fish. For example, no significant effects on the survival of *Daphnia magna* were observed following 48 hour exposures to concentrations ranging from 0.2 to 1 030 $ng \cdot L^{-1}$ (Adams et al. 1986). Likewise no adverse effects on growth or reproduction were observed when *D. magna* were exposed for up to 32 days to an average $T_4CDD$ concentration of 3.1 $ng \cdot L^{-1}$ in a contaminated mesocosm (Yockim et al. 1978). Pupation of mosquito (*Aedes aegypti*) larvae was unaffected by a 17 day exposure to 200 $ng \cdot L^{-1}$ of $T_4CDD$ (Miller et al. 1973).

Oligochaetes and snails appear to be somewhat more sensitive to $T_4CDD$ than crustaceans or

insects. Miller et al. (1973) reported that a 55 day exposure to 200 ng·L$^{-1}$ $T_4$CDD resulted in a 19% reduction in the reproductive success of the oligochaete, *Paranais* sp. This response was manifested by slower population growth and lower overall biomass in the test group relative to the control group. Similarly, a 30% reduction in reproductive success was reported in snails (*Physa* spp.) exposed to 200 ng·L$^{-1}$ for a period of 36 days (Miller et al. 1973). No adverse effects on growth or reproduction were observed in snails (*Helosoma* spp.) following a 32 day exposure to an average concentration of 3.1 ng·L$^{-1}$ (Yockim et al. 1978).

### 8.1.4 *Plants*

Aquatic plants appear to be relatively insensitive to the toxic effects of $T_4$CDD. Yockim et al. (1978) reported that long-term (32 day) exposure to $T_4$CDD levels of 3.1 ng·L$^{-1}$ had no effect on the growth or reproduction in the alga, *Oedogonium cardiacum*. Similarly, Isensee and Jones (1975) reported no adverse effects when *O. cardiacum* or duckweed *(Lemna minor)* were exposed for 33 days to $T_4$CDD concentrations of 1330 ng·L$^{-1}$ in static toxicity tests. Furthermore, no adverse effects were noted on the aquatic macrophytes, slender waterweed (*Elodea nuttalli*) and coontail (*Certophyllum emersum*) following exposure to 53.7 ng·L$^{-1}$ for several months (Tsushimoto et al. 1982).

## 8.2 Effects from PCDD/Fs in Marine Waters

No studies were found on the toxicity of PCDD/Fs on water-borne marine organisms.

## 8.3 Biochemical Responses in Fish

Biochemical effects of PCDD/F exposure have been examined in a variety of field and laboratory situations. Typically, enzyme-induction either as EROD or AHH activity has been measured although other parameters are infrequently recorded as evidenced by Spitsbergen et al (1988a) who found that both the number of leukocytes and thrombocytes were significantly reduced in the blood of juvenile rainbow trout that had been exposed to nominal $T_4CDD$ tissue doses of at least 1 $\mu g \cdot kg^{-1}$ ww. Elevated MFO induction has been correlated to increased body burden levels of PCDD/Fs in fish exposed to bleached kraft pulp mill effluents (BKME) in a number of field and laboratory studies (Hodson et al. 1992; Servos et al. 1992c; Servizi et al. 1993; Munkittrick et al. 1994; van den Heuvel et al. 1995; 1996). While PCDD/Fs are known inducers of MFO, there is growing evidence that other components of pulp mill effluent, such as naturally occurring plant steroids, may also contribute to this biochemical response in fish (Servos et al. 1992c; Munkittrick et al. 1994; van den Heuvel et al. 1995, 1996). Hence, the correlation of MFO induction to body burden levels of PCDD/Fs may be coincidental in situations where whole or diluted mill effluent is tested.

Servizi et al. (1993) exposed fingerling chinook salmon *(Oncorhynchus tshawytscha)* to various concentrations of bio-treated bleached kraft mill effluent (TBKME) at concentrations and temperatures typical of the Fraser River, BC (Table 31). Effluent samples were analyzed for contaminants including resin acids, chlorinated phenols, chlorinated guaiacols, and tetrachloro-catechol as well as PCDD/Fs throughout the study. EROD activity levels were increased approximately 2.5-fold among fish exposed to 1.5% and 4% (v/v) effluent/clean freshwater for days 1-60 and 0.3% and 8% (v/v) effluent/clean freshwater for days 61-144. After fish had been maintained in clean seawater for days 145 to 210 (representing the migration from the Fraser River to seawater), the response returned to control levels. Laboratory and field EROD data for fingerling chinook salmon from this and other studies by the same researchers analyzed jointly, showed a high linear correlation between EROD activity and TEQ body burden

in ng·kg$^{-1}$ ww ($p<0.01$, $r=0.843$, $df=270$). Servizi et al. (1993) concluded that there was an apparent threshold for MFO induction between 0.3 and 1 ng TEQ·kg$^{-1}$ ww, based on whole body tissue concentrations and TEFs from Walker and Peterson (1991). For this particular data set, the TEQs calculated using TEFs from Walker and Peterson (1991) are approximately 68% of the TEQs calculated using WHO 1998 TEFs for fish (van den Berg et al. 1998); therefore, based on WHO 1998 TEFs, the induction threshold occurs between 0.4 and 1.5 ng TEQ·kg$^{-1}$ ww. While the correlation between EROD activity and TEQ body burden does not necessarily mean that $T_4$CDD, 2,3,7,8-TCDF, 2,3,4,7,8-PCDF, and 1,3,4,8,9-PCDF (the congeners detected in the effluent) were the only inducing compounds in either the field or laboratory data, it does indicate that if these were not the only inducers, the unidentified inducers appear to correlate with them.

Hodson et al. (1992) captured fish from sites above and below a bleached kraft pulp mill on the St. Maurice River, QC and measured MFO induction, physiological effects, and tissue levels of PCDD/Fs. Liver MFO activity, measured as AHH activity, was 10-fold higher in white suckers from downstream of the mill than in upstream (control) suckers. Geometric mean concentrations of total 2,3,7,8-substituted PCDD/Fs detected in the gutted carcasses were 7.93 ng·kg$^{-1}$ ww in upstream fish compared to 206.93 ng·kg$^{-1}$ ww in fish captured immediately downstream from the mill. Conversion of these levels to TEQs using WHO 1998 TEFs for fish implies a gutted carcass tissue concentration threshold for MFO induction in white suckers between 3.33 and 26.19 ng TEQ·kg$^{-1}$ ww.

In another field study of the physiological effects of seven pulp mill effluents on white suckers, Servos et al. (1992c) reported an apparent EROD induction threshold of less than 8 ng TEQ·kg$^{-1}$ as measured in the liver, which is slightly higher than the range of 0.4 to 1.5 ng TEQ·kg$^{-1}$ (whole body) determined by Servizi et al. (1993) for juvenile chinook salmon. One of the kraft mills that Servos et al. (1992c) examined used little chlorine bleaching and while its effluent had low TEQs, the effluent still resulted in elevated EROD activity. Thus, it is likely that other components of the kraft effluent also induced EROD activity.

The results of several recent studies suggest that PCDD/Fs may not be solely responsible for the MFO induction observed in fish exposed to BKME. Pre-spawning male white collected near Jackfish Bay in Lake Superior (a site exposed to BKME) and Mountain Bay (a reference site) were caged for 2, 4, and 8 d in Blackbird Creek, a BKME receiving stream where effluent enters Jackfish Bay. These suckers did not accumulate TEQs (TEFs from Clemons et al. 1994 and Safe 1987) in their livers (van den Heuvel et al. 1995). While H4IIE bioassay-derived TEQs from Jackfish Bay suckers showed no significant differences between fish exposed for different periods of time, Mountain Bay sucker liver TEQs showed a significant, five-fold increase when fish were exposed to effluent for eight days. Recall that the H4IIE rat hepatoma bioassay measures the *in vitro* P-450-1A-inducing ability of extracts from environmental samples relative to the potency of $T_4CDD$. This method, as opposed to the direct (chemistry-derived) measurement of PCDD/Fs in environmental extracts, integrates the biological potency of a mixture of chemicals in the sample including structurally similar P-450-1A-inducing compounds [see Section 7.3]. At Jackfish Bay, van den Heuvel et al. (1996) also measured chemical and bioassay TEQs in white sucker livers over a four year period during which the pulp mill began secondary treatment of effluent. While process and treatment improvements appeared to be successful at reducing the levels of PCDD/Fs found in white sucker as evidenced by the significant reductions in analytical and bioassay TEQs in livers during summer and fall sampling periods, relative MFO induction did not decrease over the study period, suggesting that PCDD/Fs were not the dominant MFO-inducing compounds in the effluent. Similarly, Munkittrick et al. (1994) performed a survey of receiving-water environmental impacts associated with discharges from twelve Canadian pulp mills that used chlorine or sulfite kraft processes and variable treatment for effluents and found that although white suckers collected near bleached kraft mills exhibited the highest hepatic EROD induction and dioxin levels, elevated enzyme activity was also observed in fish from sites that did not use chlorine. Moreover, they also noted that elevated EROD activity was seen even at mills that had secondary treatment for their effluent and that substantial dilution of apparently nontoxic effluent did not appear to eliminate this response.

Finally, Raymond and Shaw (1997) measured EROD activity and $T_4CDD$ residues in livers of mountain whitefish and peamouth chub from the Fraser River Basin and found that while both measurements were elevated downstream of pulp mills and urban centres, dioxin levels were low, near, or below detection limits.

Based on the collective findings outlined above, studies which evaluated MFO induction from pulp mill effluents should be interpreted cautiously with respect to the assessment of threshold effect concentrations (TECs) for enzyme induction attributable to PCDD/F exposure. From the evidence presented above, it can be concluded that TECs for MFO induction determined from exposure to pulp mill effluents are confounded by the contribution of enzyme-inducing compounds that are either unknown or do not belong to the PCDD/F family. Laboratory data relating EROD and P-450-1A enzyme induction in fish to exposure to single PCDD/F congeners (i.e., not in mixture or as effluent) are available and are appropriate for assessing TECs.

Parrott et al. (1995) exposed rainbow trout (200 g) to graded doses of PCDD/Fs ($T_4CDD$, $P_5CDD$, 1,2,3,6,7,8-HCDD, 1,2,3,4,7,8-HCDD, $H_7CDD$, $T_4CDF$, $P_5CDF$ or 1,2,3,4,7,8-HCDF) via oral intubation and measured hepatic EROD activity. The TECs for EROD induction ranged from 16 pg $T_4CDD \cdot g^{-1}$ ww liver to 350 pg $H_7CDD \cdot g^{-1}$ ww liver with TECs for the other congeners tested intermediate between these two values. Conversion of the data to TEQs yields a lowest TEC of <0.001 μg $TEQ \cdot kg^{-1}$ ww liver for $H_7CDD$ and a maximal TEC of 0.020 μg $TEQ \cdot kg^{-1}$ ww liver for 1,2,3,4,7,8-HCDD. Data on the enzyme response of carp (*Cyprinus carpio*) to ip injections of graded doses of $T_4CDD$, $P_5CDD$, 1,2,3,6,7,8-HCDD, OCDD, $T_4CDF$, $P_5CDF$ or OCDF are also available. van der Weiden et al. (1994) found that the lowest TECs for hepatic EROD induction ranged from 63.8 pg $P_5CDD \cdot g^{-1}$ ww liver to 1 961 pg 1,2,3,6,7,8-$HCDD \cdot g^{-1}$ ww liver for 54-89 g fish while smaller fish (38-70 g) displayed a TEC for $T_4CDD$ of 339.5 $pg \cdot g^{-1}$ ww liver. Carp appear to require higher doses of PCDD/Fs for EROD induction to occur than rainbow trout as evidenced by the range of TECs on a TEQ basis; for 54-89 g fish, TECs ranged from 0.0196 μg $TEQ \cdot kg^{-1}$ ww liver for 1,2,3,6,7,8-HCDD to

0.1476 μg TEQ·kg$^{-1}$ ww liver for $P_5CDF$. van der Weiden et al. (1994) also measured P-450-1A protein concentrations in the carp livers and TECs ranged from 0.1221 μg TEQ·kg$^{-1}$ ww liver (for 1,2,3,6,7,8-HCDD) to 0.6355 μg TEQ·kg$^{-1}$ ww liver (for $P_5CDD$) in 54-89 g fish.

## 8.4 Effects From PCDD/Fs in Sediment

Waterborne toxicity tests are used to evaluate the toxicity of dissolved PCDD/F to aquatic organisms. Sediments, however, are a complex matrix and are generally heterogeneous in terms of their physical, chemical, and biological characteristics. Sediment-associated organisms may be exposed to PCDD/Fs that is bound to particulate matter as well as that which is dissolved in the interstitial and/or overlying waters. Consequently, separate tests are required to determine the effects of sediment PCDD/Fs on benthic organisms.

Spiked-sediment toxicity tests (SSTTs) can provide quantifiable relationships between responses of test organisms and chemical concentrations in sediments under controlled laboratory conditions. No SSTTs were located which met the CCME (1995) screening criteria for guideline development. Nonetheless, the following study is included in the current discussion of sediment-associated PCDD/Fs and the effects on benthic organisms. Barber et al. (1998) tested the effects of sediments (TOC = 1.8%) spiked with $T_4CDD$ on the marine amphipod, *Ampelisca abdita* during a 10-d whole-sediment bioassay. In this toxicity test, $T_4CDD$ concentrations in the test sediment following spiking were measured to be 0.007, <0.001, 1.1, 3.8, 10 and 25 μg·kg$^{-1}$. Survival and growth of the amphipods relative to the controls were not affected at any test concentration.

Numerous other studies have evaluated the toxicology and chemistry of field-collected sediments that contain a mixture of sediment-associated chemicals (e.g., Call et al. 1991; Ingersoll et al. 1992; Bedard and Petro 1997; Crane and Schubauer-Berigan 1997; Jaagumagi and Bedard 1997).

These studies provide evidence for associations between exposure to chemicals in sediments and adverse effects on biota. Such data reflect the interactive effects of chemical mixtures as well as the influence of various factors (e.g., sediment particle size and organic carbon content) on the bioavailability and toxicity of chemicals. These studies are field-based (although some laboratory manipulation of field-collected sediments may be a part of the study), assess sediments with a range of concentrations of PCDD/Fs and other chemicals, may include a number of test sites, may test a variety of organisms and toxicological endpoints, and may include measurements of the physicochemical characteristics of the sediment and/or interstitial and overlying waters. Toxicological endpoints commonly measured in laboratory bioassays of sediments collected in such studies primarily include mortality, development of deformities and impaired development of sexual maturity of benthic organisms. Alternatively, benthic invertebrate taxa may be enumerated and various indicators of the health of benthic invertebrate communities, such as abundance, may be reported.

Although effects of individual chemicals on benthic organisms are difficult to infer from field studies, associations between chemical concentrations and biological effects can be identified. The degree to which concentrations of an individual chemical are associated with observed effects can be determined by examining the difference in concentrations between sites at which effects are observed and those at which no effects are observed. As described in CCME (1995), concentrations associated with non-toxic, reference or control conditions can be described as having no effect. There are several instances in which the concentrations of the chemical of concern, in this case PCDD/Fs, can also be described as having no effect. These include: where there is little or no concordance between chemical concentrations and the occurrence of effects (i.e., when chemical concentrations differ only slightly between toxic or non-toxic sites, or when concentrations are actually higher at non-toxic sites), or where chemical concentrations are the same at toxic and non-toxic sites. In such cases, the observed effect is likely associated with the presence of other chemicals or other unmeasured factors; however, when concentrations of the chemical of concern are substantially higher (i.e., by a factor of two or greater) at toxic sites, the

concentration may be described as being associated with an effect.

Data from a number of field studies have been compiled and evaluated in this manner for the purpose of deriving Canadian SQGs as described further in Section 10. The following examples illustrate the use of field data in assessing associations, according to the above procedure, between sediment-associated PCDD/F concentrations and benthic community or toxicological endpoints. TEQs were calculated using WHO 1998 TEFs for fish and include PCDD/F congeners only (van den Berg et al. 1998).

Ingersoll et al. (1992) summarized information collected from benthic surveys of several areas in the Great Lakes basin, including the Buffalo and Saginaw rivers and Indiana Harbour. Mean TEQ concentrations in the collected sediments ranged from 0.0014 to 214 $\mu g \cdot kg^{-1}$. Relative densities of those species present were used to assess the health of benthic communities. For example, low abundance of Chironomidae was observed at sites in the Saginaw River with a mean TEQ concentration in the sediment of 0.23 $\mu g \cdot kg^{-1}$. In comparison, the mean TEQ concentration in sediments where high abundance of Chironomidae was observed to be 0.046 $\mu g \cdot kg^{-1}$. A TEQ concentration of approximately 0.2 mg $kg^{-1}$ may therefore be considered to be associated with, and contributing to, the observed low abundance of these benthic invertebrates.

In other studies, the toxicity of field-collected sediments has been assessed using sediment bioassays. When chemical concentrations in the sediments are also measured, associations can be established between those concentrations and the toxicological endpoint. For example, sediments collected from Canagagigue Creek, ON, were assessed for their potential toxicity to an amphipod *(Hexagenia limbata)*, midge larvae *(Chironomus tentans)*, and fathead minnow (Jaagamagi and Bedard 1997). Mean TEQ concentrations in groups of samples ranged from 0.012 to 0.18 $mg \cdot kg^{-1}$. Endpoints measured in bioassays of collected sediments included survival and growth. Field-collected sediments that significantly reduced these endpoints were deemed

'toxic'. For example, sediments containing a mean TEQ concentration of 0.18 μg $kg^{-1}$ were significantly toxic (i.e., 50% mortality) to *H. limbata* after 21 days exposure (Jaagumagi and Bedard 1997). In comparison, sediments with a mean TEQ concentration of 0.012 μg·$kg^{-1}$ were not significantly toxic (i.e., 0% mortality) to *H. limbata* in the same bioassay (Jaagumagi and Bedard 1997).

Similar types of toxicological information are available for marine sediments. For example, Windom et al. (1993) evaluated the toxicity of sediments collected from Brunswick Harbour Entrance, Georgia, to benthic invertebrates including mysids *(Mysidopsis bahia)*, and sea urchins *(Arabacia punctulata)*. Mean TEQ concentrations in the sediments ranged from 0.00056 to 0.0049 μg·$kg^{-1}$. Significant mysid mortality (i.e., 16%) and a significant reduction of normal development of sea urchins (i.e., 14.6% normal development) were associated with mean TEQ concentrations in sediments of 0.0045 and 0.0049 μg·$kg^{-1}$, respectively. In comparison, no significant effect on mysid mortality (i.e., 6.33%) or normal sea urchin development (i.e., 88.2%) was observed in sediments with mean TEQ concentrations of 0.031 and 0.00056 μg·$kg^{-1}$, respectively. This study, similarly to the freshwater studies discussed above (and data from other marine and freshwater studies that have been compiled for the derivation of SQGs as outlined in Section 10), provides additional evidence that TEQ concentrations in sediments are associated with effects on exposed organisms.

While SSTTs can be used to establish cause-and-effect relationships between PCDD/F concentrations and biological responses, a limited amount of this type of information exists for PCDD/Fs. Field studies provide evidence for associations between TEQ concentrations in sediment and observed toxicological endpoints in the presence of chemical mixtures. Both types of studies provide insights into the toxicity of sediment-associated PCDD/Fs to benthic organisms. Information on the toxicity of sediment-associated PCDD/Fs (including results from field studies and SSTTs) is required to derive Canadian SQGs for PCDD/Fs, as described in Section 10.

## 8.5 Summary of Toxicity to Aquatic Organisms

To summarize, of all species, the available data on sensitive and toxic endpoints indicate that rainbow trout have yielded the most sensitive responses in terms of LOELs to PCDD/F exposure. On a tissue residue basis for LOELs, lake trout are nearly as sensitive as rainbow trout to PCDD/F exposure when mortality is compared as the endpoint. Nonetheless, the LOEL of 0.038 ng TEQ·$L^{-1}$ for growth (after 28 days exposure) and for mortality (for 28 days exposure and 28 days depuration) in juvenile rainbow trout was the most sensitive LOEL found in the literature as a result of water-borne exposure to PCDD/Fs (Mehrle et al. 1988). Similarly, the most sensitive LOEL based on a nominal tissue dose/measured tissue residue was 0.027 µg TEQ·$kg^{-1}$ ww (for $T_4CDF$) (Mehrle et al. 1988). Finally, biochemical effects of PCDD/F exposure are confounded when complex environmental mixtures (pulp mill effluents) are assessed, due to the presence of enzyme-inducing compounds other than PCDD/Fs, but laboratory studies indicate that the lowest TEC for hepatic EROD induction occurred at <0.001 µg TEQ·$kg^{-1}$ ww liver (for $H_7CDD$) in rainbow trout (200 g) (Parrott et al. 1995).

# 9. WATER QUALITY GUIDELINES FOR THE PROTECTION OF AQUATIC LIFE

Canadian Water Quality Guidelines (WQG) for the Protection of Aquatic Life were derived according to the formal protocol developed by the CCME (1991a). In this approach, the lowest observable effect level (LOEL) is divided by an uncertainty factor to estimate long term exposure level that protects the most sensitive life stage of the most sensitive species from adverse effects. Sufficient information is available to recommend a full freshwater quality guideline. As marine data is lacking, the freshwater guideline was adopted as an interim marine guideline. Discussion of the tissue residue based equilibrium partitioning approach (TRB-EqPA) for deriving water quality values lends support to the uncertainty factor chosen for the guideline derivation procedure. These derivation procedures are detailed below.

## 9.1 CCME Protocol

The goal of a Canadian Water Quality Guideline is to protect all life stages during an indefinite exposure to a given compound found in water. As such, all components of the aquatic ecosystem (e.g., algae, macrophytes, invertebrates, fish) are considered where data exist and a single maximum value based on a long-term no-effect concentration is recommended. The protocol for the derivation of Canadian Water Quality Guidelines for the protection of aquatic life has been previously published (CCME 1991a). In short, guidelines are preferably derived from the lowest-observable-effect level (LOEL) from a chronic study using a non-lethal endpoint for the most sensitive life stage of the most sensitive aquatic species investigated; the LOEL must be statistically significant. The LOEL is multiplied by an appropriate uncertainty factor to arrive at the guideline value. The use of an uncertainty factor takes into account differences in sensitivity to a chemical variable due to differences in species, laboratory versus field conditions, and test endpoints. Where chronic data is limited or lacking, guidelines can be derived from acute studies

by converting short-term median lethal or median effective concentrations ($LC_{50}$, $EC_{50}$) to long-term no-effect concentrations (CCME 1991a).

The adverse effects of PCDD/Fs on aquatic organisms are summarized in Chapter 8. Freshwater fish exhibit a wide range of sensitivities to PCDD/Fs, however, of all species, rainbow trout yielded the most sensitive responses in terms of LOELs to PCDD/F exposure (Table 31). The LOEL of 0.038 ng TEQ·$L^{-1}$ for growth (after 28 days exposure) and for mortality (for 28 days exposure and 28 days depuration) in juvenile rainbow trout (Mehrle et al. 1988) was the most sensitive LOEL found in the literature for endpoints measured as a result of water-borne exposure to PCDD/Fs. This study is considered to be a chronic toxicity assessment since the duration of the exposures encompassed a partial or full life-cycle and steady-state body burdens of $T_4CDD$ were not achieved. Therefore, in accordance with CCME protocol (CCME 1991a) the most sensitive LOEL of 0.038 ng TEQ·$L^{-1}$ is multiplied by an uncertainty factor of 0.001, to result in a Canadian WQG for the protection of freshwater aquatic life of 0.038 pg TEQ·$L^{-1}$. This uncertainty factor was chosen for two main reasons. First, an uncertainty factor of 0.01 is typically chosen for persistent substances in the environment (CCME 1991a). Considering half-lives in various media (see Chapter 4), PCDD/Fs are persistent as defined by the Toxic Substances Management Policy (Environment Canada 1997). Second, that dioxins and furans also readily accumulate in aquatic biota ($BCF_{lipid}$ = 175 245) justifies the use of an additional level of safety to account for bioconcentration. The TRB-EqPA for water was used to determine to how large the additional safety margin should be. As described below, an additional factor of 0.1 (for a total uncertainty factor of 0.001) provides sufficient protection to freshwater life from chronic exposure of PCDD/Fs. The WQG for freshwater is also comparable to water quality values set by other jurisdictions (see Section 9.3).

Insufficient toxicological data were available to support the derivation of a water quality guideline for the protection of marine and estuarine aquatic life. Thus, the freshwater guideline of 0.038 pg TEQ·$L^{-1}$ is adopted as an interim marine/estuarine guideline until sufficient data

become available.

## 9.2 Supporting Evidence - Tissue Residue Based Equilibrium Partitioning Approach (TRB-EqPA)

As described above, the formal protocol was used for the development of water quality guidelines (CCME 1991a). The discussion below outlines the tissue residue based equilibrium partitioning approach (TRB-EqPA) for water that was used to determine to how large the additional safety margin needed to be to account for the bioaccumulation of PCDD/Fs. In this method, a water quality value is calculated from a threshold effect concentration (TEC) in tissue and a bioconcentration factor (BCF).

As reviewed in Section 5.1, bioconcentration refers to the direct uptake of a substance from water across the gill membrane by aquatic organisms and is different from bioaccumulation which also takes into account dietary uptake. BCFs describe the magnitude of this process and are a measure of the ratio between the concentration of a substance in an aquatic organism and the concentration in the water. Through the use of a suitable BCF and a threshold effect concentration (TEC, in tissue), it is possible to estimate, by back-calculation, a TEQ concentration in the water that is not expected to result in adverse effects in aquatic life. Essentially, this type of an approach assumes that equilibrium partitioning processes between the biotic phase (tissues of aquatic organisms) and the abiotic phase (water) will occur. The derivation of TEQ level in water using this approach requires two values: i) a lipid-normalized TEC in tissue that represents a level above which adverse effects would be expected to occur, and ii) a laboratory-derived, lipid-normalized, steady-state BCF. Division of the TEC by the BCF should yield a concentration in water that will not result in the bioconcentration of PCDD/Fs to harmful levels in the tissues of aquatic organisms. Additional information on TEC and BCF values is provided in the following sections of this chapter.

The underlying impetus for using this method in which water and sediment quality values are determined by back-calculation from a TEC in aquatic tissues is threefold. Firstly, a TEC is an additional assessment tool by which to measure potential adverse effects of a substance in environmental media such as water or sediments. This is particularly true of hydrophobic, bioaccumulative substances that readily partition into the organic fraction of the environment. For instance, although sediments may act as an important source for the transfer of PCDD/Fs into the aquatic food chain and are generally regarded as a reservoir for these substances in aquatic systems, they often contain PCDD/Fs at limits which are near or below detection capabilities (Fletcher and McKay 1993). Similarly, due to the strong hydrophobicity and partitioning behaviour of PCDD/Fs, concentrations in environmental water samples are also near or below detection limits (Grimwood and Dobbs 1995). Therefore, it is not always possible to define effect versus no-effect levels.

Secondly, fundamental toxicological theory advocates that exposure water concentration is only a surrogate for the toxic dose (or 'effective' dose) within the body of the organism, and further, that the whole-body residue is itself a substitute for the amount of toxic substance at the site(s) of toxic action (Filov et al. 1979). The exposure water (or sediment) concentration may only be reliably employed when the relationship between the exposure medium and the body residue is known and clearly understood (McCarty et al. 1992; Walker and Peterson 1994a).

Thirdly, several factors exist that may affect the potential for a toxic substance to exert adverse biological effects on aquatic biota. TECs have inherent consideration of factors that tend to vary among species, including, a) bioavailability of the toxic substance, b) source of uptake, c) effects of metabolism on accumulation, and d) accumulation kinetics (McCarty and Mackay 1993).

### 9.2.1 *Threshold Effect Concentration (TEC) in Tissue*

The steps to derive the TEC in tissue that was used in the calculation of the water-based value are detailed below. A TEC based on hepatic EROD induction (i.e., the minimum dose causing statistically significant increases in EROD activity above controls) was chosen based on the data from Parrott et al. (1995) that focused on responses to various PCDD/Fs in 200 g rainbow trout. The steps taken to derive the particular TEC in tissue that will be used in the calculation of the water-based value are detailed below. Foremost, it may be argued that an MFO induction threshold (i.e., a TEC based on EROD induction) may be a more appropriate measure to use with respect to effect characterization than the lowest LOEL value (based on tissue concentration for other sensitive or toxic endpoints) because the enzyme induction represents a conservative limit (see Section 7.1.2). As stated earlier, MFO induction alone does not necessarily indicate the triggering of a harmful effect, but PCDD/Fs apparently cannot evoke a toxic response unless MFO induction occurs. To date, enzyme induction is the most sensitive marker to indicate that an adverse biological effect or toxic response may transpire. Thus, a TEC based on EROD induction should provide an acceptable safety margin for the calculation of a concentration in water of PCDD/Fs that will not cause adverse effects in aquatic organisms. The TEC should also be on a lipid weight rather than a wet weight basis because the mean $BCF_{lipid}$ that will be used is based on lipid weight. Finally, the TEC based on EROD induction should not include data derived from studies where organisms were exposed to bleached kraft mill effluent (BKME). As mentioned in Section 8.3, recent evidence suggests that the role of PCDD/Fs in causing MFO induction in fish exposed to BKME is questionable.

The laboratory studies of Parrott et al. (1995) indicate that the lowest TEC for hepatic EROD induction occurred at <0.001 μg $TEQ^{-1}$ ww liver (for $H_7CDD$) in rainbow trout. A TEC based on the lowest TEC is deemed to be unnecessarily conservative in consideration of the safety margin already established through the use of a TEC based on enzyme induction rather than a toxic endpoint. Thus, a geometric mean TEC was calculated to represent an overall threshold effect

level. The lowest LOEL based on a nominal tissue dose/measured tissue residue of 0.027 μg TEQ·kg$^{-1}$ ww (for $T_4$CDF) for mortality in juvenile rainbow trout determined by Mehrle et al. (1988) was set as the upper limit above which TECs for EROD induction should not be included in the calculation of the geometric mean TEC. Using an estimated lipid fraction of 5% (cited from Schmieder et al. 1995) for 0.38 g rainbow trout, the LOEL (0.027 μg TEQ·kg$^{-1}$ ww) can be converted to a lipid weight basis resulting in an LOEL for mortality of 0.540 μg TEQ·kg$^{-1}$ lipid. The TECs for hepatic EROD induction for 38 to 89 g carp measured by van der Weiden et al. (1994; see Table 31) ranged from 0.0638 μg TEQ·kg$^{-1}$ ww for $P_5$CDD to 0.0196 μg TEQ·kg$^{-1}$ ww for 1,2,3,6,7,8-HCDD corresponding to 0.8 to 29.7 μg TEQ·kg$^{-1}$ lipid (from van der Weiden et al. 1994), respectively. Clearly, these TECs are above the 0.540 μg TEQ·kg$^{-1}$ lipid upper limit and were not included in the calculation of the mean TEC. The TECs determined by Parrott et al. (1995) on a lipid weight basis, were however, all lower than this limit and were all included in the calculation. The TECs for hepatic EROD induction for $T_4$CDD, $H_6$CDD, $H_7$CDD, $T_4$CDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF, and $H_6$CDF (0.016, 0.020, <0.001 set at 0.001, 0.002, 0.002, 0.0024, and 0.005, respectively) converted to a lipid weight basis using an average lipid content in liver of 28% (J.L. Parrott, Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, Ontario, pers. com.) resulted in corresponding TECs of 0.057, 0.071, 0.004, 0.007, 0.007, 0.0086, and 0.018 μg TEQ·kg$^{-1}$ lipid, respectively. Subsequently, the geometric mean TEC from these numbers is 14 ng TEQ·kg$^{-1}$ lipid. This value, converted to a wet-weight basis (assuming a 5% whole body lipid level; Schmieder et al. 1995), is 0.7 ng TEQ·kg$^{-1}$, a value nearly identical to the tissue residue guideline (TRG) of 0.71 ng TEQ·kg$^{-1}$ for the protection of wildlife consumers of aquatic biota derived herein (see Chapter 12).

### 9.2.2 *Bioconcentration Factor (BCF)*

The $BCFs_{lipid}$ recorded for $T_4$CDD were the highest of all congeners and, therefore, may be used

as a conservative measure to estimate uptake of PCDD/Fs from water only (not including uptake from food or sediment exposure). The geometric mean $BCF_{lipid}$ for $T_4CDD$ calculated from the steady-state (or estimates thereof) data for resident fish species is 175 245 (see Section 5.1 and Table 12). This value integrates species differences, differential exposure conditions (i.e., variable water concentrations, dissolved organic carbon levels in the water etc.), various life stages, and reflects different analytical methods of determining BCFs. The latter factor refers to the treatment of the water samples when estimating the water concentrations of $T_4CDD$. Specifically, whether samples were whole (i.e., unfiltered), filtered, or passed through cartridges designed to measured the truly 'dissolved' $T_4CDD$. The mean $BCF_{lipid}$ reflects only freshwater conditions as data pertaining to marine/estuarine environments were not located in the literature. The mean $BCF_{lipid}$ was calculated on a lipid-weight basis to remove variability attributable to lipid content. It is generally accepted that the size of the lipid pool in an organism will affect the concentration, expressed on a wet weight or dry weight basis, of lipophilic compounds. PCDD/Fs are highly lipophilic substances and accumulate in fatty tissues. For example, Servos et al. (1994) reported that, on a wet-weight basis, the concentration of PCDD/Fs in the liver tissue of white suckers (*Catastomus commersoni*) was up to 45 times greater than in the muscle fillet of the same fish. This difference was eliminated if the concentrations were normalized for the lipid contents of each tissue type. Finally, the mean $BCF_{lipid}$ of 175 245 incorporates only data from studies where uptake of $T_4CDD$ into aquatic organisms was unequivocally due to uptake from water only and not through ingestion of contaminated food or sediment. Field-derived 'BCFs' (or BAFs), although reflecting real world processes, including uptake from food and/or sediment, may introduce site-specific or local variability into a comparison of uptake into aquatic organisms. Another advantage to evaluating laboratory-derived BCFs as opposed to field-derived BAFs is that steady state conditions (or lack thereof) may be readily assessed while this is difficult or impossible with field investigations.

### 9.2.3 *Water Quality Value calculated from TRB-EqPA*

Dividing the mean TEC of 14 ng TEQ·kg$^{-1}$ lipid by the mean $BCF_{lipid}$ of 175 245 results in a water based value of 0.08 pg TEQ·L$^{-1}$ that is not expected to result in adverse effects in freshwater life. This value provides support for an additional margin of safety for the value derived using the CCME protocol approach.

## 9.3 Water Quality Values from other Jurisdictions

The U.S. EPA has reported acute and chronic lowest observed effect levels for $T_4CDD$ of 10 ng·L$^{-1}$ and 10 pg·L$^{-1}$, respectively (U.S. EPA 1990). More recently, the U.S. EPA (1993) reported low risk water levels of $T_4CDD$ for the protection of fish of 0.6 to 3.1 pg·L$^{-1}$, depending on the level of particulate organic carbon in the water.

A guideline of 10 pg·L$^{-1}$ for the protection of the aquatic ecosystem in the Great Lakes has frequently been ascribed to the International Joint Commission (IJC). This value originated from a recommendation made by the IJC (1980) that $T_4CDD$ should be (analytically) absent (i.e., not detectable) from all compartments of the ecosystem. At that time, the detection limit for this substance in water was 10 pg·L$^{-1}$. The National Wildlife Federation and the Canadian Institute for Environmental Law and Policy (NWF and CIELP 1991) recommends that the water quality standard for $T_4CDD$ in the Great Lakes should be no more that 0.0067 pg·L$^{-1}$. This level was recommended to protect wildlife from adverse effects associated with the consumption of $T_4CDD$-contaminated aquatic organisms. In 1995, the U.S. EPA recommended a more protective value of 0.0031 pg·L$^{-1}$ as the Great Lakes Wildlife Criterion for $T_4CDD$ (U.S. EPA 1995).

## 9.4 Canadian Water Quality Guidelines

A Canadian Water Quality Guideline (WQG) of 0.038 pg TEQ·$L^{-1}$ for total PCDD/Fs is recommended for the protection of freshwater life. This value was calculated by applying an uncertainty factor of 0.001 to a LOEL of 0.038 ng TEQ·$L^{-1}$ for growth (after 28 days exposure) and for mortality (for 28 days exposure and 28 days depuration) in juvenile rainbow trout (Mehrle et al. 1988). As no data were located on the toxicity of PCDD/Fs to marine or estuarine organisms, the freshwater quality guideline of 0.038 pg TEQ·$L^{-1}$ for total PCDD/Fs is adopted as the interim water quality guideline for the protection of marine and estuarine biota, until sufficient data are available to derive an effects-based guideline.

This guideline is designed to protect aquatic organisms from the toxic effects of water-borne PCDDs and PCDFs, as well as against the adverse effects which may be associated with the bioconcentration of these substances directly from the water. The value applies to the TEQ concentration due to PCDD/Fs in whole water samples. It is recommended that sediment quality and tissue residue guidelines be used in conjunction with these values to ensure that aquatic organisms are protected from adverse effects associated with accumulation via benthic-based food chains.

Although much smaller than older 10 pg·$L^{-1}$ criteria (IJC 1980; U.S. EPA 1990), this guideline value of 0.038 pg·$L^{-1}$ is approximately ten-fold larger than more recent values (0.0031 and 0.0067 pg·$L^{-1}$) proposed for the protection of wildlife in the Great Lakes Region (NWF and CIELP 1991; U.S. EPA 1995). Further, as evidenced by the TRB-EqPA for water, the guideline value of 0.038 pg·$L^{-1}$ is justified by the high $BCF_{lipid}$ for $T_4CDD$ (175 245) and by the threshold effect concentration (TEC) for tissue (14 ng·$kg^{-1}$ lipid). Moreover, the TEC, when converted to a wet weight basis (0.7 ng·$kg^{-1}$), is nearly identical to the TRG for wildlife (0.71 ng·$kg^{-1}$), suggesting that TEQ toxic threshold for fish applies also to the wildlife that consume them.

### 9.4.1 *Data Gaps*

There is a paucity of PCDD/F toxicity data for marine organisms. To upgrade the Canadian Water Quality Guideline for the protection of marine and estuarine biota from an interim to full guideline status, the minimum data requirements set forth in the CCME Protocol must be met (CCME 1991a). At least three primary toxicity studies on temperate marine fish species are required. At least two of these studies must be a subchronic or chronic (partial or full life cycle test). For invertebrates, at least two chronic (partial or full life cycle test) studies on two or more temperate marine species from different classes are needed. And, at least one study on a temperate marine vascular plant or marine algal species is required. For both freshwater and marine organisms, little information was located on the toxicity of individual congeners other than $T_4CDD$. Few studies were located on the effects of PCDD/F mixtures with the exception those which tested BKME. Non-additive effects and interactions among laterally and non-laterally substituted congeners need to be further elucidated.

# 10. SEDIMENT QUALITY GUIDELINES FOR THE PROTECTION OF AQUATIC LIFE

The production of PCDD/Fs has resulted in the widespread distribution of these substances in the Canadian environment and elevated PCDD/Fs concentrations in sediments from a number of locations. As discussed earlier, exposure of benthic organisms to sediment-associated PCDD/Fs may result in uptake of, and adverse biological effects associated with, these substances (see Sections 5.2 and 8.4). Effective assessment of the potential ecological impact of PCDD/Fs in sediments requires an understanding of the relationships between sediment-associated concentrations of PCDD/Fs and the occurrence of adverse biological effects. National sediment quality guidelines are scientific tools that synthesise information regarding such relationships in a form that is easily communicated and understood. In Canada, they are developed using toxicological information and are intended to be national benchmarks (i.e., reference points) to be used in making decisions regarding the protection of specified resource uses (e.g., aquatic life).

In Canada, the CCME has developed a formal protocol to derive numerical sediment quality guidelines (SQGs) for both freshwater and marine (including estuarine) sediments to protect aquatic life associated with bed sediments (CCME 1995). This protocol relies on the National Status and Trends Program (NSTP) approach (with modifications) and the Spiked-Sediment Toxicity Test (SSTT) approach. Subsequent to an evaluation of the toxicological information, Canadian SQGs (also referred to as 'full' SQGs) are recommended if information exists to support both approaches. Generally, the lower of the two values derived using either the NSTP approach or SSTT approach is recommended. (It should be noted that, while sufficient data currently exist to calculate SSTT values for several substances, concerns regarding spiked-sediment toxicity testing methodology limit the degree to which these values may be used as the scientific basis for recommending full SQGs at this time). If insufficient information exists to derive interim guidelines using either the modified NSTP approach or the SSTT approach,

guidelines from other jurisdictions are evaluated and may be provisionally adopted in the short-term as interim SQGs. Interim Canadian SQGs (ISQGs) are recommended if information is available to support only one approach. The guidelines may also be derived to reflect predictive relationships that have been established between the concentration of the chemical in sediments and any environmental factor or condition (e.g., characteristics of the sediment, such as the concentration of organic carbon; characteristics of the overlying water column, such as hardness) involved in modifying the expression of the toxicity of the chemical.

Details of the derivation and evaluation of Canadian SQGs for PCDD/Fs are outlined in the following sections. At present, insufficient information exists to derive ISQGs for PCDD/Fs using the SSTT approach. Interim SQGs for PCDD/Fs in freshwater sediments have been derived using the modified NSTP approach. Discussion of the tissue residue based equilibrium partitioning approach (TRB-EqPA) for deriving sediment quality values lends support to the uncertainty factor chosen for the freshwater ISQG. These derivation procedures are detailed below. Because there was insufficient information available to derive ISQGs for PCDD/Fs in marine/estuarine sediments using the modified NSTP approach, the ISQG derived for freshwater sediments has been adopted for marine/estuarine sediments in the short term as a provisional ISGQ. CCME (1995) should be consulted for a detailed description of the protocol and its supporting rationale.

## 10.1 The Modified National Status and Trends Program (NSTP) Approach

The NSTP approach to deriving sediment quality assessment values was originally developed by Long and Morgan (1990). This approach was adopted, with some modifications, for use in developing SQGs in Canada (CCME 1995) and is therefore referred to in this document as the *modified* NSTP approach. Modifications to this approach include the separate evaluation of information for freshwater and marine systems, an expansion of the information originally compiled, and the use of derivation procedures that consider all of the compiled information (see

also Long and MacDonald 1992; MacDonald 1994; CCME 1995; and MacDonald et al. 1996).

The modified NSTP approach primarily relies on the use of field-collected data, in which chemical mixtures occur, to derive sediment quality assessment values (Long and Morgan 1990; Long 1992; Long and MacDonald 1992; MacDonald 1994; CCME 1995; Long et al. 1995). This approach involves the evaluation and compilation of sediment quality data from numerous studies conducted throughout North America, in which sediment chemical and biological data have been collected synoptically (also referred to as co-occurrence data). Data from individual studies are evaluated to establish an *association* between the concentration of each of the chemicals measured in the sediments and any adverse biological effect observed. Cause-and-effect relationships between the concentration of individual chemicals and the observed adverse biological effect cannot be inferred from this evaluation. This information is compiled in a database referred to as the Biological Effects Database for Sediments (BEDS).

Sediment chemical and biological data currently included in BEDS were obtained from various studies, including models of equilibrium partitioning in sediments, sediment quality assessment values from other jurisdictions, spiked-sediment toxicity tests, and field studies (including acute and chronic toxicity results from sediment bioassays and analyses of benthic community composition). Candidate studies were critically evaluated according to a number of screening criteria (e.g., appropriate sediment handling procedures, acceptable toxicity test procedures) to ensure that high quality data sets were incorporated into BEDS and that the information compiled was internally consistent (MacDonald 1994; CCME 1995; Long et al. 1995; Environment Canada 1996).

Information contained in BEDS was sorted for each chemical and sediment type (i.e., freshwater versus marine) and arranged in ascending order of the chemical's concentration to produce separate ascending data tables, or guideline derivation tables, for individual chemicals and sediment types (MacDonald 1994; CCME 1995; Long et al. 1995). These tables summarise the

compiled information that associates a chemical's concentration with either adverse effects or no adverse effects on aquatic organisms (also referred to as the effect data set and the no-effect data set, respectively). Data in BEDS pertaining to PCDD/Fs are presented here in Tables 32 and 33. Concentrations of PCDD/Fs are expressed as μg TEQ·kg$^{-1}$ dw, which were calculated using the latest TEF values for fish (van den Berg et al. 1998). The following discussion provides a general overview of the guideline derivation tables assembled in BEDS.

The guideline derivation tables for individual chemicals (e.g., PCDD/Fs; Tables 32 and 33) consist of a number of entries, each of which belongs to either the effect data set or the no-effect data set, that are sorted according to ascending chemical concentrations (for PCDD/Fs these are TEQ-based). Entries in the guideline derivation tables were designated as being associated with an effect (an asterisk in the 'Hit' column) if an adverse biological effect was reported. These effects included acute or chronic toxicity observed during a controlled spiked-sediment test, apparent effect thresholds (concentrations above which specific biological effects would always be expected), and predicted toxicity based on equilibrium partitioning theory (which determines a sediment chemical concentration that ensures the concentration of the chemical in the interstitial water does not exceed the water quality guideline for that chemical at equilibrium). An entry was also assigned an asterisk (effect descriptor) if concordance was apparent between the observed biological response and the measured chemical concentration in a field (i.e., co-occurrence) study. Concentrations of individual chemicals reported in field studies were considered to be associated with the observed toxic response (i.e., concordance was apparent) if the mean concentration at sites at which significant adverse effects were observed was a factor of two or more greater than the mean concentration at sites at which effects were not observed (i.e., at toxic versus non-toxic sites) (Long et al. 1995). A factor of two was chosen to ensure that the difference in the response (i.e., adverse effect) was associated with a significant difference in the chemical concentration. For each chemical, all of the entries designated by an asterisk, as described above, are collectively referred to as the effect data set.

All entries in the guideline derivation tables (e.g., Tables 32 and 33) other than those designated by an asterisk are collectively referred to as the no-effect data set, and are represented by those entries for which chemical concentrations were not associated with adverse biological effects. These entries include those associated with non-toxic, reference, or control conditions (i.e., no effects; NE), as well as those for which toxicity may have been observed but the mean chemical concentration differed by less than a factor of two between the toxic and non-toxic groups (i.e., no gradient, NG; small gradient, SG; or no concordance, NC). In the latter case, it was assumed that other factors (whether measured or not) were more important in the etiology of the observed effect than the chemical concentration (Long et al. 1995).

Individual entries in the guideline derivation tables consist of the chemical concentration in the sediment, an indication of whether this concentration was part of the effect data set (i.e., *) or the no-effect data set (i.e., NE, SG, NG, or NC), location of the study, analysis type (or approach used), test duration, toxicological endpoint measured, species and life stage tested, information on the characteristics of the sediments (e.g., concentrations of TOC or AVS, physical classification of the sediments; when available), and the study reference. In most cases, information for individual entries (i.e., chemical concentrations and sediment chemical and physical characteristics) represents the means of several samples. Standard deviations of these means are provided wherever possible. The following discussion summarises the information contained in the freshwater and marine guideline derivation tables for PCDD/Fs (Tables 32 and 33, respectively).

### 10.1.1 *Guideline Derivation Tables for PCDD/Fs*

Mean concentrations of sediment-associated PCDD/Fs range from 0.0083 to 214 μg TEQ·kg$^{-1}$ and from 0.00056 to < 100 μg TEQ·kg$^{-1}$ in the freshwater (Table 32) and marine (Table 33) guideline derivation tables, respectively. A number of adverse biological effects are associated with sediment concentrations of PCDD/Fs ranging from 0.0011 to 2.1 μg TEQ·kg$^{-1}$ in freshwater

sediments and from 0.0012 to < 100 μg TEQ·kg$^{-1}$ in marine sediments.

The freshwater guideline derivation table for PCDD/Fs (Table 32) contains 82 entries, a major proportion of which are results from field (Co-occurrence Approach; COA) studies (73%). The remaining data (11%) are sediment quality assessment values from other jurisdictions that have been derived using a variety of approaches, including the Equilibrium Partitioning Approach (EqPA), and the Sediment Background Approach (SBA). The marine guideline derivation table (Table 33) contains 19 data entries, with the majority of entries from field studies (84%). The remaining data (16%) are sediment quality assessment values from other jurisdictions, including spiked sediment toxicity values (Spiked Sediment Bioassay Approach; SSBA) and equilibrium partitioning values.

Amphipoda, Chironomidae, and Ephemeroptera are the most common freshwater taxa for which toxicological information was obtained and included in the freshwater guideline derivation table, whereas Gastropoda, Oligochaeta and fish are less frequently represented. A number of benthic taxa are represented in the marine guideline derivation table, including Echinodermata, Amphipoda, Mollusca, Polychaeta and Crustacea. In addition, entries are included for a single fish species *(Menidia berllina)*. In both the freshwater and marine guideline derivation tables, mortality and changes in benthic invertebrate abundance are the most common indicators of adverse biological effects measured in field (COA) studies.

Information on total organic carbon (TOC), acid volatile sulphide (AVS), unionised ammonia, and the physical classifications of the bed sediments is also included for each entry in the guideline derivation tables, when available (Tables 32 and 33). It should be noted, however, that individual entries in the ascending data tables represent means of several samples; therefore ranges for characteristics of sediments summarised below may be narrower than the ranges of these variables reported in the original studies from which the tables were derived. Nonetheless, these ranges provide an indication of the variety of sediment types represented in the ascending

data tables. For example, the mean TOC content of sediments ranges from 0.2 to 10.0% and from 0.1 to 2.8% in the freshwater and marine tables, respectively. The mean AVS concentration ranges from 2.6 to 44.4 $\mu mol \cdot g^{-1}$ in the freshwater studies and from 16.3 to 14 009 $\mu mol \cdot g^{-1}$ in the marine studies. The few studies that measured unionised ammonia in interstitial waters reported values ranging from 0.02 to 0.82 $\mu g \cdot L^{-1}$ and from 0.25 to 18.25 $\mu g \cdot L^{-1}$ in freshwater and marine samples, respectively. The mean proportions of sand, silt, and clay in the freshwater sediments represented in the ascending data table range from 2.8 to 98.2%, 1.5 to 64.8%, and 2.3 to 83.3%, respectively. The mean proportions in marine sediments, reported from a single study, were 51.6% for sand, 20.2% for silt, and 28.2% for clay. As the range for each size class is large, the data in these tables represent a wide variety of sediment types.

### 10.1.2 *Derivation of the TEL and PEL*

As is described in CCME (1995), the derivation procedures used to calculate two assessment values in the original NSTP approach (Long and Morgan 1990) were modified to consider both the effect and no-effect data compiled in the guideline derivation tables. In the modified NSTP approach, the lower value, referred to as the threshold effect level (TEL), represents the concentration below which adverse biological effects are expected to occur rarely. The upper value, referred to as the probable effect level (PEL), defines the level above which adverse effects are expected to occur frequently. Concentrations that fall in the range between the TEL and the PEL are occasionally expected to be associated with adverse biological effects. The definition of these ranges (also referred to as the minimal, possible, and probable effect ranges) is based on the assumption that the potential for observing toxicity of sediment-associated chemicals increases with increasing chemical concentrations (Long et al. 1995).

Minimum toxicological data requirements have been set to ensure that the TELs calculated from the guideline derivation tables provide adequate protection of aquatic life and that these values

are supported by a weight of evidence for a broad range of sediment types and characteristics. For a given guideline derivation table, both the effect data set and the no-effect data set must contain at least 20 entries (CCME 1995). These minimum data requirements have been met for the derivation of the guideline for PCDD/Fs in freshwater sediments (24 effect entries and 58 no-effect entries), but not for marine sediments (3 effect entries and 16 no-effect entries). Therefore, only the freshwater TEL and PEL have been calculated for PCDD/Fs as described below. These two assessment values refer to the concentration of PCDD/Fs (on a TEQ basis) in surficial sediments (i.e., top 0-5 cm) on a dry-weight basis.

The TEL is calculated as the geometric mean of the lower 15$^{th}$ percentile concentration of the effect data set (i.e., $E_{15}$) and the 50$^{th}$ percentile concentration of the no-effect data set (i.e., $NE_{50}$), as follows:

$$TEL = \sqrt{E_{15} \times NE_{50}}$$

The TEL for PCDD/F calculated from data in the freshwater guideline derivation table (Table 32) is:

$$TEL_{freshwater} = \sqrt{0.008 \times 0.012}$$
$$= 0.010\ \mu g\ TEQ \cdot kg^{-1}$$
$$= 10\ ng\ TEQ \cdot kg^{-1}$$

In addition to the TEL, a PEL is also calculated from the guideline derivation tables. The PEL is calculated as the geometric mean of the 50$^{th}$ percentile concentration of the effect data set (i.e., $E_{50}$) and the 85$^{th}$ percentile concentration of the no-effect data set (i.e., $NE_{85}$), as follows:

$$PEL = \sqrt{E_{50} \times NE_{85}}$$

The PEL calculated from data in the freshwater guideline derivation table (Table 32) is:

$$PEL_{freshwater} = \sqrt{0.185 \times 0.194}$$
$$= 0.189 \mu g\ TEQ \cdot kg^{-1}$$
$$= 189\ ng\ TEQ \cdot kg^{-1}$$

### 10.1.3 *Evaluation of the TEL and PEL*

The objective of establishing the TEL and PEL according to a standard formula, as described above, is to consistently define the range of chemical concentrations within which adverse effects rarely occur (i.e., the minimal effect range; below the TEL) and within which adverse biological effects frequently occur (i.e., the probable effect range; above the PEL), respectively. The definition of the TEL is therefore consistent with the definition of a Canadian SQG. The PEL, in contrast, is an additional tool that can be used in conjunction with the SQG, and other relevant information, in assessing sediment quality.

The incidence of adverse biological effects below the TEL (i.e., within the minimal effect range) and above the PEL (i.e., within the probable effect range), can be used to evaluate the degree to which the TEL and PEL satisfy their narrative objectives (MacDonald 1994; CCME 1995; Smith et al. 1996a, b). The incidence of effects within each range is quantified by dividing the number of effect entries by the total number of entries in that range and expressing this ratio as a percentage. The TEL and PEL calculated from a guideline derivation table are considered to meet their objectives when the incidence of effects below the TEL is less than or equal to 25% and the incidence of effects above the PEL is greater than or equal to 50%, respectively (MacDonald 1994; Smith et al. 1996a).

In the freshwater guideline derivation table, the incidence of adverse biological effects associated

with PCDD/F concentrations below the TEL is 17% (Figure 3). The low incidence of adverse biological effects observed below the TEL suggests that the TEL for freshwater sediments adequately represents a level below which effects are expected to occur rarely. Sediment PCDD/F concentrations that fall below 0.010 $\mu g\ TEQ \cdot kg^{-1}$ (or 10 $ng\ TEQ \cdot kg^{-1}$) dry weight would not be expected to be associated with adverse biological effects. The incidence of adverse effects between the TEL and the PEL, and above the PEL increases to 27% and 50%, respectively (Figure 3). Concentrations that fall in the range between the TEL and the PEL are occasionally expected to be associated with adverse biological effects (CCME 1995). The freshwater PEL for PCDD/Fs meets the narrative objective (i.e., $\geq$ 50 % incidence of adverse effects above the PEL), which suggests the PEL for freshwater sediments adequately represents a level above which effects are expected to occur frequently.

A detailed analysis of the guideline derivation tables indicates that the data from which the TEL and PEL for freshwater sediments were derived represents a limited number of locations, sediment types, sediment characteristics, species, life stages, and biological endpoints in comparison to guideline derivation tables for other chemicals. Ideally, incorporation of data from a broad range of geographic locations in the derivation process increases the likelihood that the TEL and PEL will be broadly applicable and national in scope. Data on field-collected sediments reflect variation in biological responses associated with organic carbon content, AVS concentrations, interactions among chemicals, and other measured or unmeasured factors that may influence the occurrence of biological effects associated with PCDD/Fs. Although the way in which any such variation affects the occurrence of effects in the field is difficult to quantify, it is implicitly incorporated, as described above, in the calculation of the TEL and PEL and in the incidence of effects in the concentration ranges defined by them. Likewise, utilisation of information on a wide range of sediment-resident species, on a variety of life stages, and on several experimental endpoints ensures that the TEL and PEL adequately reflect the range of biological responses that could be observed in association with PCDD/F concentrations in sediments.

## 10.2 The Spiked-Sediment Toxicity Test (SSTT) Approach

The SSTT approach, which involves an independent evaluation of information from spiked-sediment toxicity tests (some of which are included in the guideline derivation tables described above), is a complementary procedure to the modified NSTP approach for estimating the concentration of a chemical in sediments below which adverse effects are not expected to occur (CCME 1995). In contrast to the modified NSTP approach, which is used to derive TELs and PELs using information on *associations* between chemical concentrations in sediments and effects on sediment-associated organisms, the SSTT approach is used to derive SSTT values using data from controlled laboratory tests in which organisms are exposed to sediments that have been spiked with known concentrations of a chemical. Such studies provide quantifiable cause-and-effect relationships between the concentration of a chemical in sediments and the observed biological response (e.g., survival, reproductive success, growth). Spiked-sediment toxicity tests are generally used to evaluate the toxic effect of a single chemical, or specific mixture of chemicals, to exposed organisms. They may also be used to determine the extent to which environmental conditions modify the bioavailability of a chemical, and ultimately the response of organisms exposed to the spiked-sediments.

Minimum toxicological data requirements have been set for the SSTT approach to ensure that SSTT values derived using spiked-sediment toxicity information provide adequate protection to aquatic organisms (CCME 1995). Data requirements include at least four independent studies on two or more sediment-resident invertebrate species that occur in North American waters. For freshwater sediments, at least one species must be a benthic crustacean and one must be a benthic arthropod (other than a crustacean). For marine sediments, at least one species must be a benthic amphipod. Spiked-sediment toxicity test values can be derived from studies conducted on sensitive species (e.g., fish, aquatic plants, protozoa, fungi, bacteria) provided that the minimum

data set requirements are met. An additional requirement is that at least two of the studies must be partial or full life-cycle tests that consider ecologically-relevant endpoints (e.g., growth, reproductive success, developmental effects). In addition to these requirements, the procedures used to generate spiked-sediment toxicity data must be evaluated and determined to be appropriate before they can be used to develop SSTT values. Although methods (e.g., spiking procedures, equilibration periods) for spiked-sediment toxicity tests are currently not standardised (Environment Canada 1995a), information provided in individual studies (e.g., equilibrium time, stability of concentrations over the test duration, responses in control treatments) can be used to evaluate the influence of the use of specific methods on test results.

Insufficient data exist to derive SSTT values for PCDD/Fs in either freshwater or marine sediments. However, evaluations of available spiked-sediment toxicity tests are discussed in Section 8.4.

## 10.3 Supporting Evidence

As described above, the formal protocol was used to derive a TEL for freshwater sediments (CCME 1995). Because PCDD/Fs are persistent in sediment as indicated by their half-lives in this media (see Chapter 4) and because they readily accumulate in aquatic biota, two approaches were informally examined to determine whether the TEL for freshwater sediment would be adequately protective aquatic life. These approaches included the water-sediment Equilibrium Partitioning Approach (EqPA) and the Tissue Residue-Based Equilibrium Partitioning Approach (TRB-EqPA) for sediment. The discussion below details the derivation of sediment quality assessment values using these two approaches.

### 10.3.1 *Water-Sediment Equilibrium Partitioning Approach (EqPA)*

The equilibrium partitioning approach (EqPA) may be used to calculate a sediment quality assessment value (SQAV) or a concentration of a substance in sediments that ensures the concentration in the interstitial water does not exceed the water quality guideline for that chemical, at equilibrium (U.S. EPA 1992). A SQAV that is intended to protect freshwater or marine aquatic life can be derived using the EqPA and appropriate Canadian WQG. SQAVs derived using this approach are based indirectly on biological effects (i.e., only to the extent that the WQG used in its calculation is effects-based). An EqPA based SQAV may be derived using the following equation:

$$SQAV = WQG \cdot K_p$$

where

SQAV = Sediment Quality Assessment Value ($ng \cdot kg^{-1}$)

WQG = Canadian Water Quality Guideline for the protection of aquatic life ($ng \cdot L^{-1}$)

$K_p$ = water/sediment partition coefficient ($L \cdot kg^{-1}$)

where

$K_p = K_{oc} \cdot f_{oc}$

$K_{oc}$ = organic carbon/water partition coefficient ($L \cdot kg_{oc}^{-1}$)

$f_{oc}$ = fraction of organic carbon in the sediment ($kg_{oc} \cdot kg^{-1}$)

Although there are many factors that influence the partitioning of sediment-associated chemicals between the sediment and interstitial water phases, the fraction of organic carbon ($f_{oc}$) in sediment is believed to be the most important factor influencing the bioavailability of non-ionic hydrophobic organic chemicals when total organic carbon is greater than 0.5% (SAIC 1991).

Under these conditions, $K_p$ (i.e., the water/sediment partition coefficient) can be estimated by multiplying the fraction of organic carbon ($f_{oc}$) in the sediment by the organic carbon/water partition coefficient ($K_{oc}$) (Karickhoff 1981, Mackay 1991). Organic carbon/water partition coefficients may be obtained from the literature where they have been measured in lab (experimental, i.e., batch shaking in flasks) or field situations or estimated using simple linear relationships between $K_{oc}$ and $K_{ow}$ (i.e., $K_{oc} = 0.41\ K_{ow}$; Karickhoff 1981). Table 6 summarises $K_{oc}$s for 2, 3, 7, 8-substituted PCDD/Fs. Only measured (i.e., either in lab or field situations) $K_{oc}$s are included in this table, as $K_{oc}$s estimated from $K_{ow}$s would introduce unnecessary variability into the calculation of a mean $K_{oc}$ (i.e., error propagation would occur because each estimation would inherently have variability included in its choice of constant and $K_{ow}$ value). It is apparent that information on congeners other than $T_4CDD$ is limited. Therefore, for the purposes of deriving a sediment quality assessment value using the EqPA, a geometric mean $K_{oc}$ of 827 000 was calculated from the values summarised for $T_4CDD$.

The recommended interim Canadian WQG for freshwater life is 0.038 pg TEQ·$L^{-1}$ (see section 9.4). Using this freshwater WQG, an $f_{oc}$ of 1%, and the geometric mean $K_{oc}$ of 827 000, an EqPA based $SQAV_{freshwater}$ for PCDD/Fs is calculated, as follows:

$$SQAV_{freshwater} = 0.000038 \text{ ng TEQ}\cdot L^{-1} \cdot 827\,000 \text{ L}\cdot kg_{oc}^{-1} \cdot 0.01\ kg_{oc}\cdot kg^{-1}$$
$$= 0.314 \text{ ng TEQ}\cdot kg^{-1}$$

As the WGQ for freshwater was adopted as the interim marine WQG, the EqPA based SQAV for marine sediments would also be 0.314 ng TEQ·$kg^{-1}$. These EqPA based SQAVs are almost two orders of magnitude less than the TEL for freshwater sediments (10 ng·$kg^{-1}$) derived using the modified NSTP approach (see Section 10.1.2).

### 10.3.2 *Tissue Residue Based Equilibrium Partitioning Approach (TRB-EqPA) for Sediment*

The discussion below outlines the tissue residue based equilibrium partitioning approach (TRB-EqPA) for deriving sediment quality assessment values, which involves the establishment of a 'safe' concentration of the chemical in sediment that is expected to result in an acceptable tissue residue in aquatic organisms (U.S. EPA 1992; Iannuzzi et al. 1995). This method is based on the Biota-Sediment Accumulation Factor (BSAF) (also known as the Bioavailability Index, BI), with the assumption that non-polar organic chemicals will partition from organic carbon in the sediments to the lipids in aquatic organisms. Through the use of a suitable BSAF and a threshold effect concentration (TEC, in tissue), it is possible to estimate, by back-calculation, a PCDD/F concentration in sediment that is not expected to result in adverse effects in aquatic life. Essentially, this type of an approach assumes that equilibrium partitioning processes between the biotic phase (tissues of aquatic organisms) and the abiotic phase (sediment) will occur. A comparable TRB-EqPA for water was used as supporting evidence for the derivation of the Canadian WQG for the protection of freshwater life. Please refer to Section 9.2 for an explanation of the rationale behind using the TRB-EqPA approach.

As describe above, a SQAV may be calculated by dividing a lipid-normalised TEC in tissue by an appropriate BSAF. A TEC representing a level above which adverse effects would be expected to occur has been previously derived in Section 9.2.1. Overall BSAFs were calculated separately for freshwater and marine/estuarine environments (see Table 13 and Section 5.2). Briefly, because the degree of variability in the BSAFs appeared to outweigh the error associated with a particular method of estimation, both experimental data (laboratory and field-derived data were combined for each type of environment) and BSAFs calculated based on non-detectable concentrations (set at detection limits) were included in the determination of overall BSAFs. The data for guppies and European eels were not included in the calculation as these species do not occur in Canadian waters. Biota-sediment accumulation factors for Rice Creek, Florida, however, were included in the calculation of the overall BSAF for marine/estuarine ecosystems

because the species sampled are also found in Canadian waters. The geometric mean BSAF, representing an overall BSAF for freshwater environments calculated from the available data, is 0.30, while the overall BSAF for marine/estuarine environments is 0.14 (see Section 5.2).

Therefore, sediment concentrations that are predicted to result in tissue concentrations at or below the TEC can be estimated from the equation:

$$SQAV = (TEC \div BSAF) \cdot f_{oc}$$

Where:

TEC = threshold effect concentration (ng TEQ·kg$^{-1}$ lipid)

BSAF = geometric mean biota-sediment accumulation factor [(lipid)·(OC)$^{-1}$] (see Section 5.2 )

$f_{oc}$ = fraction of organic carbon in the sediment ($kg_{oc}$·kg$^{-1}$)

Assuming a TEC of 14 ng TEQ·kg$^{-1}$ lipid (see Section 9.2.1), a freshwater BSAF of 0.30 (see Section 5.2), and an organic carbon fraction of 1%, the freshwater SQAV is calculated as follows:

$$SQAV_{freshwater} = (14 \div 0.30) \cdot 0.01$$
$$= 0.47 \text{ ng TEQ·kg}^{-1}$$

Assuming a TEC of 14 ng TEQ·kg$^{-1}$ lipid (see Section 9.2.1), a marine BSAF of 0.14 (see Section 5.2), and an organic carbon fraction of 1%, the marine SQAV is calculated as follows:

$$SQAV_{marine} = (14 \div 0.14) \cdot 0.01$$
$$= 1.0 \text{ ng TEQ·kg}^{-1}$$

The TRB-EqPA based $SQAV_{freshwater}$ is comparable to the EqPA based SQAVs (freshwater and

marine) of 0.314 ng TEQ·kg$^{-1}$, but is smaller by approximately 20 fold in comparison to the TEL for freshwater sediments (10 ng·kg$^{-1}$) derived using the modified NSTP approach. In comparison, the TRB-EqPA based $SQAV_{marine}$ is an order of magnitude less than the TEL.

## 10.4 Sediment Quality Values from other Jurisdictions

Several jurisdictions have proposed numerical sediment quality assessment values for PCDD/Fs for either freshwater or marine systems. The United States and The Netherlands have developed assessment values to protect aquatic organisms from adverse effects associated with exposure to PCDD/Fs in sediments. For example, the U.S. EPA (1993) calculated that there was a low risk of observing adverse effects on fish when freshwater sediment $T_4CDD$ concentrations were at or below 60 ng·kg$^{-1}$ dw (3% TOC; or 20 ng·kg$^{-1}$ at 1% TOC). The corresponding high risk value for fish is 100 ng·kg$^{-1}$ dw (3% TOC; 33 ng·kg$^{-1}$ at 1% TOC). The New York Department of Environmental Conservation (NYSDEC) proposed an assessment value for $T_4CDD$ of $<1.0 \times 10^7$ ng·kg$^{-1}$ OC (or $<1.0 \times 10^5$ ng·kg$^{-1}$ at 1% TOC) to protect aquatic organisms (NYSDEC 1994). This value was derived using an equilibrium partitioning approach and the state water quality standard for aquatic toxicity. The Netherlands calculated Maximum Tolerable Risk levels (MTRs), based on equilibrium partitioning theory, of 378 ng TEQ·kg$^{-1}$ (calculated using I-TEFs) for the protection of sediment organisms (Liem et al. 1993).

Sediment quality assessment values to protect uses (e.g., wildlife) in addition to aquatic organisms have been established in both the United States and the Netherlands. The New York State Department of Environmental Conservation proposed a sediment quality assessment value for $T_4CDD$ of 200 ng·kg$^{-1}$ OC (or 2 ng·kg$^{-1}$ at 1% TOC) to protect wildlife (using equilibrium partitioning theory and an ambient water quality guidance value for fish-eating mammals; NYSDEC 1994). Another sediment quality assessment value was proposed by NYSDEC (1989) of 3 to 30 ng·kg$^{-1}$, which was derived from a $T_4CDD$ wildlife fish flesh criterion. The U.S. EPA

(1993) calculated low risk levels for the protection of mammals and fish-eating birds for $T_4CDD$ of 2.5 and 21 $ng \cdot kg^{-1}$, respectively (at 3% TOC). The corresponding high risk values for mammals and fish-eating birds are 25, and 210 $ng \cdot kg^{-1}$ (at 3% TOC), respectively. The Netherlands determined Maximum Tolerable Risk levels (MTRs) to be 15 ng $TEQ \cdot kg^{-1}$ (calculated using I-TEFs) for the protection of predators of sediment organisms (Liem et al. 1993). In addition, the Netherlands developed otter-based sediment quality objectives for TEQs based on the health and physiological effects of PCBs in otters, and on information from case-studies on PCB bioaccumulation and biomagnification (Smit et al. 1996). Safe and critical levels were calculated based on $EC_1$ and $EC_{90}$ values for vitamin A deficiency, respectively. The safe TEQ concentration in sediment was determined to be 3 ng $TEQ \cdot kg^{-1}$ OC, (i.e., 0.2 ng $TEQ \cdot kg^{-1}$ dw); the critical concentration was determined to be 7 ng $TEQ \cdot kg^{-1}$ OC (i.e., 0.4 $ng \cdot kg^{-1}$ dw).

Sediment quality assessment values to protect ecological receptors and human health have also been proposed by several jurisdictions. The International Joint Commission (IJC) recommended a freshwater sediment quality guideline of 10 ng $T_4CDD \cdot kg^{-1}$ dw for the protection of both ecological and human receptors in the Great Lakes ecosystem (IJC 1980). This guideline was based on analytical detection limits. The New York State Department of Environmental Conservation proposed three sediment quality assessment values for $T_4CDD$ with respect to the protection of human health. Using an equilibrium partitioning approach and the New York ambient water quality standard, a sediment quality value of 10 000 $ng \cdot kg^{-1}$ OC (or 100 $ng \cdot kg^{-1}$ at 1% TOC) was proposed (NYSDEC 1994). In addition, sediment quality assessment values for $T_4CDD$ of 10.0 to 100 $ng \cdot kg^{-1}$ and 0.014 to 0.14 $ng \cdot kg^{-1}$ were proposed as a human health fish consumption advisory and a cancer risk for fish consumption, respectively (NYSDEC 1989).

## 10.5 Canadian Sediment Quality Guidelines

### 10.5.1 *Freshwater Sediments*

Sufficient toxicological data exist for freshwater sediments to use the modified NSTP approach to develop a freshwater TEL and PEL. Insufficient toxicological data exist to calculate a freshwater SSTT value using the SSTT approach. According to the formal protocol (CCME 1995), a TEL calculated using the modified NSTP approach may be recommended as an ISQG. An evaluation of the incidence of effects observed below the freshwater TEL and above the freshwater PEL indicates that the narrative objectives for these values have been met according to the formal protocol. Notwithstanding, PCDD/Fs may persist in sediments for long periods (Chapter 4; OMOE 1985) and have a capacity for bioaccumulation (Chapter 5). A detailed assessment of the guideline derivation tables indicates that the data may not adequately address these aspects. For example, the number of chronic studies is under-represented, and toxicity tests that have been compiled were commonly conducted with invertebrate species with relatively short life spans that may not be exposed to contaminated sediments for long periods of time. In comparison to guideline derivation tables for other chemicals, those tables for PCDD/Fs have fewer entries and appear to be not as representative of a diverse body of evidence regarding effects of sediment associated PCDD/Fs. Furthermore, the SQAVs developed using EqPA and TRB-EqPA approaches were at least an order of magnitude less than the TEL, indicating that the TEL may not adequately protect aquatic organisms. Therefore use of a safety factor of 0.1 is recommended to account for these uncertainties, and to achieve a better estimate of the concentrations of sediment-associated PCDD/Fs that will not harm aquatic organisms associated with bed sediments over an indefinite period of exposure.

The recommended interim freshwater sediment quality guideline (ISQG) for PCDD/Fs is, therefore, 1 ng TEQ·kg$^{-1}$ dw. This guideline is recommended for PCDD/Fs concentrations in freshwater surficial sediments (i.e., top 0-5 cm).

In order to derive a full freshwater SQG for PCDD/Fs, at least four independent spiked-sediment toxicity studies on North American freshwater sediment-resident invertebrates that meet the CCME screening criteria are required. These SSTT studies must include at least one benthic crustacean species and one benthic arthropod species (other than a crustacean). In addition, at least two of the four studies must include partial or full life-cycle tests that consider ecologically-relevant endpoints (e.g., growth, reproduction, developmental effects; CCME 1995). Because concerns exist regarding sediment spiking methods (Environment Canada 1995a), advances in methods standardisation and/or the development of methods performance criteria is also required before such data could be used as the basis for the recommendation of a full freshwater SQG for PCDD/Fs. Existing evidence is sufficient to determine that the ISQG (=TEL·UF) is applicable to a wide range of sediment types. Therefore, for the purposes of national SQG development in Canada, it was not necessary to adjust the TEL for variation in sediment characteristics. Nonetheless, additional studies should examine relationships between the bioavailability and toxicity of sediment-associated PCDD/Fs and characteristics of the sediment and overlying water column at specific sites. The results of such studies would assist in refining the means by which such factors are considered in the site-specific implementation of freshwater SQGs for PCDD/Fs.

The freshwater PEL of 189 ng TEQ·kg$^{-1}$ dw was adjusted to 18.9 ng TEQ·kg$^{-1}$ dw, by applying the same uncertainty factor (0.1) as was applied to the TEL, in order to account for uncertainties discussed above. This PEL is recommended as an additional sediment quality assessment tool.

The recommended ISQG for PCDD/Fs in freshwater sediments is not comparable to philosophically-similar sediment quality guidelines recommended by other jurisdictions (see Section 10.4). The guidelines proposed by the Netherlands (378 ng TEQ·kg$^{-1}$) and the state of New York (<1.0 X 10$^{5}$ ng·kg$^{-1}$ at 1% TOC) for the protection of aquatic organisms are much higher than the freshwater ISQG.

Because the TEL and PEL are derived from existing toxicological data, they do not specifically consider adverse effects on higher trophic levels which may occur as a result of the bioaccumulation of PCDD/Fs in exposed organisms. These values indirectly consider bioaccumulation to the extent that the expression of adverse biological effects include effects of bioaccumulation (i.e., many effects occur because toxic substances are taken up and accumulate in organisms; Smith et al. 1996b). Other procedures are required to derive sediment quality guidelines that will specifically protect higher trophic levels. Sediment quality assessment values proposed by other jurisdictions to protect other ecological receptors (i.e., wildlife in the food web) ranged from 0.2 $ng \cdot kg^{-1}$ dw to 70 $ng \cdot kg^{-1}$ at 1% TOC, with the majority of the values falling between 0.2 $ng \cdot kg^{-1}$ dw and 20 $ng \cdot kg^{-1}$ at 1% TOC. In comparing these values to the recommended freshwater ISQG for the protection of aquatic organisms, it appears that the freshwater ISQG may also provide some degree of protection to higher organisms in the food web that are exposed to PCDD/Fs.

### 10.5.2 *Marine/Estuarine Sediments*

Insufficient toxicological data exist for marine sediments to use the modified NSTP approach to develop a marine TEL and PEL, or to calculate a marine SSTT value using the SSTT approach. In the absence of toxicological information to support either of these approaches, sediment quality assessment values from other jurisdictions are normally considered for adoption as a provisional Canadian ISQG in the short-term (CCME 1995). The freshwater sediment ISQG is the most representative of current information that incorporates data on the biological effects of PCDD/Fs on aquatic organisms. Furthermore, from an evaluation of the existing toxicity data, there is no indication that sensitivities differ among freshwater or marine organisms. Therefore, the ISQG for freshwater sediments, 1 ng $TEQ \cdot kg^{-1}$ dw, is the provisionally recommended ISQG for marine/estuarine surficial sediments (i.e., top 0-5 cm) for PCDD/Fs.

In order to derive a marine ISQG for PCDD/Fs using the modified NSTP approach, both the effect and no-effect data sets must meet the minimum data requirements of at least 20 data entries each for deriving a TEL and PEL (CCME 1995). Alternatively, independent spiked-sediment toxicity studies on North American marine or estuarine sediment-resident invertebrates are required to derive an ISQG using the SSTT approach (CCME 1995). Because no spiked-sediment toxicity data currently exist that meet the screening criteria of CCME (1995), at least four independent spiked-sediment toxicity studies on North American marine sediment-resident invertebrates are required. At least one of these must be a benthic crustacean species, and one must be benthic arthropod species (other than a crustacean). Furthermore, at least two of the studies must be partial or full life-cycle tests that consider ecologically-relevant endpoints (e.g., growth, reproduction, developmental effects).

In the short-term, the adjusted freshwater PEL of 18.9 ng TEQ·kg$^{-1}$ dw is recommended as an additional assessment tool for evaluating the quality of marine sediments, until such time as sufficient information is available to derive a marine PEL using the modified NSTP approach. It is anticipated that the adjusted PEL will be useful in identifying sediments in which adverse biological effects are more likely to occur (i.e., in sediments with PCDD/F concentrations above the PEL).

The Canadian ISQGs and adjusted PELs for PCDD/Fs developed in this report are scientific benchmarks (i.e., reference points) that can be used, along with other relevant tools and information, as the basis for evaluating, protecting, and enhancing sediment quality. Although these sediment quality guidelines are considered interim at this time, they should not be used differently than they would be if they were full sediment quality guidelines. Moreover, these Canadian Sediment Quality Guidelines should be use in conjunction with guidelines for other media (e.g., water and tissue), as well as guidelines for other compounds (e.g., PCBs).

# 11. TOXICITY TO MAMMALIAN AND AVIAN SPECIES

As with other media, the most widely studied PCDD/F congener in mammalian and avian species is $T_4CDD$. Reports for other congeners are limited, but where available, are summarised in the text and tables. In such cases, concentrations have been converted to TEQ concentrations using WHO 1998 TEFs (van den Berg et al. 1998). Otherwise, the source of the TEFs used by the original author(s) is given.

Studies specifically designed to test enzyme induction (e.g., MFO enzymes such as cytochromes P-450-1A1 and 1A2) were not included in the evaluation of studies of the development of the TRG for dioxins and furans. A thorough review of enzyme induction has been recently completed for the purpose of deriving the latest WHO TEF values (van den Berg et al. 1998).

## 11.1 Toxicity to mammals

### 11.1.1 *Acute*

Acute sensitivities of mammalian receptors to $T_4CDD$ span four orders of magnitude (Table 34). Guinea pigs are most sensitive to acute exposure with a reported $LD_{50}$ of 0.6 $\mu g \cdot kg^{-1}$ bw (Schwetz et al. 1973). In contrast, single dose oral $LD_{50}$s for male and female Golden Syrian hamsters reportedly vary from 1157 to 5051 $\mu g \cdot kg^{-1}$ bw (Olson et al. 1980; Henck et al. 1981). The latter value is believed to be more accurate, as determination of the former was complicated by ileitis, a condition which presumable made the hamsters more susceptible to $T_4CDD$ toxicity (Henck et al. 1981). Regardless, Golden Syrian hamsters appear to be the least sensitive mammalian species to the lethal effect of $T_4CDD$. Rats ($LD_{50}$ = 22 to 45 $\mu g \cdot kg^{-1}$ bw), rabbits ($LD_{50}$ = 115 $\mu g \cdot kg^{-1}$ bw), and mice ($LD_{50}$ = 114 to 284 $\mu g \cdot kg^{-1}$ bw) appear to be moderately sensitive to the effects of $T_4CDD$ (Schwetz et al. 1973; Vos et al. 1974). Few data exist on the acute toxicity

of $T_4CDD$ to wildlife species that consume aquatic biota; however, mink may be among the most sensitive species ($LD_{50}$ = 4.2 $\mu g \cdot kg^{-1}$ bw; Hochstein et al. 1988). For many species, mortality due to single doses of $T_4CDD$ is delayed (i.e., 5 to 45 d; U.S. EPA 1987).

In addition to mortality, decreased body weight gain and/or food consumption have been observed in guinea pigs, rats, and mink (Greig et al. 1973, Hochstein et al. 1988). For example, Hochstein et al. (1988) reported significant decreases (11%) in the body weights in mink (28 d post-exposure) following a single administration of 2.5 $\mu g \cdot kg^{-1}$ bw of $T_4CDD$. Other symptoms of acute toxicity may include depletion of adipose tissue, ulcerations of the stomach, mottled and discoloured livers and kidneys, and changes in relative organ weights (DeCaprio et al. 1986; Hochstein et al. 1988; Shara and Stohs 1987).

In contrast to more sensitive species, mice and hamsters do not appear susceptible to weight loss when treated with single doses of $T_4CDD$. For example, there were no significant affects on body weight gain in pregnant albino mice given single doses of up to 90 $\mu g \cdot kg^{-1}$ (Nag et al. 1993). Hamsters generally gained weight following $T_4CDD$ treatment, although a dose dependent decrease in body weight occurred three weeks following treatment of high doses (1000-6000 $\mu g \cdot kg^{-1}$ bw; Henck et al. 1981). Hanberg et al. (1990) reported an $ED_{50}$ for growth in hamsters of > 1000 $\mu g$ $T_4CDD \cdot kg^{-1}$ bw. Hamsters that die as a result of $T_4CDD$ toxicity display signs of wasting syndrome with loss of adipose stores and muscle mass; moreover, target organs, namely liver and thymus, appear to be the same as those for more sensitive species (Olson et al.1980; Henck et al. 1981).

Neither 2,7-DCDD, 1,2,4- $T_3CDD$, 1,2,3,4-TCDD, nor OCDD produced body weight loss, thymic atrophy, or induced lipid peroxidation, or inhibited glutathione peroxidase in female rats dosed 40 or 400 $\mu g \cdot kg^{-1}$ bw for three days. None of these congeners induced *Ah* activity in rats similarly dosed (Shara and Stohs 1987).

Alterations to normal physiological functions in mammalian receptors are commonly associated with exposures to $T_4CDD$. In some species, changes in liver morphology (e.g., necrosis, proliferation of endoplasmic reticulum, etc.) and size have been reported following administration of acutely toxic doses of $T_4CDD$ (U.S. EPA 1987; Birnbaum et al. 1989a, b; Hanberg et al. 1990; Lans et al. 1990). Vos et al. (1974) reported extensive haemorrhaging in mice exposed to $T_4CDD$ and suggested that this may have contributed to the ultimate death of these organisms. Still other investigators have observed that exposure of rats to $T_4CDD$ results in reduced activities in key gluconeogenic enzymes and have hypothesised that this was a possible cause of acute toxicity (Weber et al. 1991). Female rats dosed 40 $\mu g \cdot kg^{-1}$ bw for three consecutive days experienced significantly reduced heart rate and blood pressure (Hermansky et al. 1988). It is not currently known, however, to what degree any of these responses are associated with the toxic action of $T_4CDD$.

### 11.1.2 *Chronic*

Long-term oral exposure to relatively low levels of $T_4CDD$ may result in a variety of sub-lethal responses, including weight loss, hair loss, chloracne, and edema (Mukerjee et al. 1986; Table 35). In addition, a number of biochemical, physiological, and histological effects have also been observed following chronic exposure to $T_4CDD$ (Lilienfeld and Gallo 1989; SRC 1989a). Common biochemical effects include induction of specific enzyme systems (e.g., MFO) and suppression of the immunological system (see also Section 11.1.4; Fishbein 1987). Observed physiological effects include loss of fat, shrinkage of the thymus, spleen, and other lymphatic tissues, and alterations in the number of blood cells (WHO 1989; Lakshman et al. 1991). Upon histological examination of test organisms, changes in the liver and thickening of the gastrointestinal tract, the gall bladder, and the bile duct are frequently evident (OMOE 1985).

Reduced growth and liver toxicity appear to be sensitive responses in $T_4CDD$ exposed mammals.

Rats fed 0.01 and 0.1 $\mu g \cdot kg^{-1} \cdot d^{-1}$ for two years experienced increased mortality, decreased weight gain, and increased relative liver weight (Kociba et al. 1978). Those fed 0.01 $\mu g \cdot kg^{-1} \cdot d^{-1}$ had increased liver weights, but no changes in mortality or growth; there were no observable effects at the 0.001 $\mu g \cdot kg^{-1} \cdot d^{-1}$ dose. Mustonen et al. (1989) administered doses of $T_4CDD$ ranging from 0 to 0.7 $\mu g \cdot kg^{-1}$ bw$\cdot d^{-1}$ to male rats over a period of 14 d. A 15% increase in the liver somatic index was observed in the groups receiving 0.07 $\mu g \cdot kg^{-1}$ bw$\cdot d^{-1}$ or more. In a longer-term study (91 d), rats that received a daily dose of 0.0036 $\mu g$ TEQ$\cdot kg^{-1}$ bw of OCDD had a significantly higher incidence of liver lesions than control rats (Birnbaum et al. 1989b). Mice appear to be somewhat more tolerant than rats to exposure of $T_4CDD$ (U.S. Dept. of Health and Human Services 1982). In male mice, increased liver weights (13%) were observed following administration of 0.143 $\mu g \cdot kg^{-1}$ bw$\cdot d^{-1}$ of $T_4CDD$ for a period of 14 d (Vos et al. 1974).

DeCaprio et al. (1986) found that significant reductions in growth (22-39%) and increased liver weights (relative to body weights) were exhibited by both sexes of guinea pigs when weanlings were fed 4.9 ng $T_4CDD \cdot kg^{-1}$ bw$\cdot d^{-1}$ for 90 d. Reduced relative thymus weights and elevated serum triglycerides were also observed in males receiving this dose, while females exhibited hepatocellular cytoplasmic inclusion bodies and lowered serum alanine aminotransferase activities. These authors reported NOELs of 0.61 and 0.68 ng$\cdot kg^{-1}$ bw$\cdot d^{-1}$ for males and females, respectively. Although guinea pigs are the most sensitive species to acute and chronic $T_4CDD$ exposure, they display neither severe liver damage or edema that are common to many other species. Moreover, hemorrahages in guinea pigs are primarily restricted to the gastrointestinal track and the urinary bladder, while in rats, massive haemorrhages have been reported for many organs (Vos et al. 1974).

### 11.1.3 *Reproductive/Developmental*

Unlike the toxicity of PCBs, where both parent compounds and metabolites may be directly or

indirectly responsible, reproductive and developmental toxicity of $T_4CDD$ appears to be associated only with the parent compound (Peterson et al. 1993). Furthermore, one distinct effect of $T_4CDD$ that has not been observed for dioxin-like PCBs is the malformation of a cleft palate.

Numerous studies have demonstrated that $T_4CDD$ is fetotoxic and teratogenic in rodents, with effects commonly observed at doses that are not overtly toxic to the mother. In rats, symptoms of fetotoxicity (decreased growth, haemorrhage, edema, and death of the fetus) are more sensitive indicators of toxicity those of teratogenicity (cleft palate) (Table 36). For example, administration of a single oral dose of $T_4CDD$ (1.5 $\mu g \cdot kg^{-1}$ bw) to pregnant female rats on gestation day 10 resulted in significant gastrointestinal haemorrhaging in the fetuses within 10 d (Olson et al. 1990). Increases in the incidence of cleft palate (38% incidence) in fetal rats did not occur until dams were dosed 18 $\mu g \cdot kg^{-1}$ bw on gestation day 10 and was accompanied by high fetal mortality (72%) and significant decrease in maternal body weight.

With the exception of a single dam that died of pneumonic consolidation of the lungs during mating, rats receiving 0.1 $\mu g$ $T_4CDD \cdot kg^{-1}$ $bw \cdot d^{-1}$ for 90 d prior to mating displayed no evidence of clinical toxicity (Murray et al. 1979). Fertility, litter size at birth, and gestation survival, however, were significantly reduced for these dams compared to control rats (Murray et al. 1979). At 0.01 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$, fertility, pups per litter, and generation survival index among litters were significantly reduced in $f_1$ and $f_2$ but not $f_0$ generations. No significant effects on fertility, litter size, or postnatal body weight were observed in rats dosed 0.001 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$ compared to control animals in any of the three generations (Murray et al. 1979).

The reproductive system of sexually mature male rats is relatively insensitive to $T_4CDD$. For example, the $ED_{50}$ for androgen deficiency is 15 $\mu g \cdot kg^{-1}$ bw. In contrast, maternal $T_4CDD$ exposure to a concentration of 0.16 $\mu g \cdot kg^{-1}$ bw on gestation day 15 resulted in significant decreases in anogenital distance, time to testis descent and seminal vesicle weight. Doses as low

as 0.064 $\mu g \cdot kg^{-1}$ bw impaired spermatogenesis (Mably et al. 1991). Neither of these latter doses caused signs of overt toxicity in dams or their offspring. The 100 fold difference in sensitivity observed between rats exposed perinatally and sexually mature rats underlines the importance that developmental stage can play in the evaluation of toxic responses (Mably et al. 1991).

In contrast to rats, teratogenic endpoints are more sensitive in mice and are highly specific, resulting in the induction of cleft palate and hydronephrosis at doses below those causing overt maternal or fetal toxicity (Birnbaum et al. 1987; 1989a). Single dose $ED_{50}$s for cleft palate in mice are 15.6 and 11.9 $\mu g \cdot kg^{-1}$ bw when dams are treated on days 10 and 12 of gestation, respectively (Birnbaum et al. 1989a). The $ED_{50}$ for hydronephrosis is 3.9 $\mu g \cdot kg^{-1}$ bw regardless of treatment day. No effects occurred on fetal viability, mortality, or weight at doses up to 18 and 15 $\mu g \cdot kg^{-1}$ bw treated on day 10 and 12 of gestation, respectively (Birnbaum et al. 1989a). Combination treatment of $T_4CDD$ and retinoic acid (metabolite of vitamin A) resulted in an enhancement of the incidence of cleft palate but not hydronephrosis in mice (Birnbaum et al. 1989a). From a study in which pregnant mice were dosed $T_4CDD$, $T_4CDF$, or a combination of both, it was concluded that for cleft palate frequency in fetuses, $T_4CDD/F$ toxicity is additive, with one unit of $T_4CDD$ approximately equal to 30 units of $T_4CDF$ (Weber et al. 1985). Although the dose levels were too high to demonstrate a clear dose-response relationship, lesions in the fetal kidney appears to be a more sensitive endpoint than cleft palate development, which is consistent with the lower $ED_{50}$ for hydronephrosis compared to that for cleft palate (Weber et al. 1985; Birnbaum et al. 1989a).

Even though hamsters are reportedly the least sensitive species to $T_4CDD$ toxicity, an increased incidence of fetal kidney abnormalities was observed following the administration of a single oral dose of 1.5 $\mu g \cdot kg^{-1}$ bw to pregnant females (Olson et al. 1990). A single dose of 2 $\mu g \cdot kg^{-1}$ bw to female hamsters at gestation day 11.5 is sufficient to cause a 30% reduction in body weight of male offspring at 5 months of age. Core body temperatures of these offspring were also significantly reduced compared to male offspring from untreated mothers (Gordon et

al. 1996).

Nursing may pose greater risk of $T_4CDD$ toxicity to young rodents than perinatal exposure; while $T_4CDD$ crosses the placenta in rodents, exposure of the offspring occurs mainly through nursing as high levels of unmetabolized $T_4CDD$ is excreted in milk (Lucier et al. 1975, cited in Luster et al. 1980). Research on nursing effects is limited but significantly decreased body weight of neonates was reported when dams were exposed to 5.0 $\mu g \cdot kg^{-1}$ bw (or 0.5 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$) over the first 10 days of lactation (Lans et al. 1990).

Two studies that assessed the cumulative effects of dioxin-like compounds on reproduction and development in mammals were found. Mink were fed diets containing 0, 10, 20 or 40% contaminated carp from Lake Michigan prior to and throughout the reproductive period (26 weeks total). Mink consumed, on average, 0.011, 0.013, 0.012, and 0.015 $\mu g$ PCDD/Fs$\cdot kg^{-1}$ $bw \cdot d^{-1}$, or 0.23, 3.89, 7.34, and 10.2 ng TEQ$\cdot kg^{-1}$ $bw \cdot d^{-1}$, respectively, with PCDD/Fs contributing 6-32% of the TEQ concentration (Heaton et al. 1995a; TEQs re-calculated with WHO 1998 TEFs). Concentrations of organochlorine compounds other than PCDD/Fs and PCBs were minimal in diets and do not affect reproduction in mink (Heaton et al. 1995a). Females fed the 40% carp diet whelped significantly fewer kits, and all kits were either stillborn or dead within 24 h. There was a significant inverse dose-dependent response between weights of kits and proportion of carp in the maternal diet, with 20 and 40% carp diet groups significantly different from the control. Reduced kit body weights at three weeks of age occurred under the 10% carp diet and were followed by reduced survival of the kits at three and six weeks of age. Percent kit survival to six weeks of age (weaning) was 85, 28, 11.5, and 0% for the 0, 10, 20, and 40% carp diets, respectively. Relative organ weights of kits whelped and nursed by treated females were generally less than those of the control group. Liver concentrations of the adult females ranged from <10 to 656 TEQs$\cdot kg^{-1}$ (as estimated by the H4IIE bioassay; Tillitt et al. 1996).

A multigeneration study in which Sprague-Dawley rats fed 0, 2, or 20% chinook salmon from Lakes Huron and Ontario found no significant correlations among TEQ dietary intakes (up to 2.84 ng TEQ·kg$^{-1}$ bw·d$^{-1}$; I-TEFs) and mating, fertility, viability, or lactation indices, save larger litters were commonly noted among rats fed 20% fish diets (Feeley and Jordon 1998; Arnold et al. 1998; Feeley et al. 1998). The only statistically significant effect observed was that female off-spring of the first and second generations had larger relative liver weights compared to controls.

### 11.1.4 *Immune System*

Research into $T_4$CDD effects on the immune system has been almost entirely conducted on mice, despite immunosuppression being reported as a common symptom associated with $T_4$CCD exposure (Table 37). In mice, $T_4$CDD may affect both the specific and non-specific defence mechanisms of the immune system which in turn may adversely affect host defence capabilities (Holladay et al. 1991). Cellular and humoral immunity are the two main components of the specific immune response and both are susceptible to $T_4$CDD.

In cellular immunity, lymphocytes that migrate from the bone marrow to the thymus develop into T-cells. At maturity, T-cells next migrate to the lymphatic tissues (e.g., lymph nodes and spleen) where their function includes attacks on infected or defected cells. Thus, while the thymus typically atrophies in adult mammals, it is essential for normal development of the lymphatic system and immune response as a whole (Hoar 1975).

Adverse effects on the thymus, particularly thymic atrophy, have been observed in a variety of species following sublethal exposure to PCDD/Fs. Thymic atrophy ($ED_{50}$) has been reported to occur in four week old guinea pigs 28 days after administration of a single $T_4$CDD dose of 0.8 µg·kg$^{-1}$ bw (Hanberg et al. 1990). Significant reductions (39%) in the relative thymus

weights of mice pups were also observed when dams were administered daily doses of 1.5 $\mu g \cdot kg^{-1}$ bw on gestation days 6 to 14 (Holladay et al. 1991). Hamsters are least susceptible to thymic atrophy with an $ED_{50}$ value of >1000 $\mu g$ $T_4CDD \cdot kg^{-1}$ bw (Hanberg et al. 1990). Rats fed diets contaminated with $T_4CDD$, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, or a PCDD/F mixture derived from flyash experienced reduced thymus weights at TEQ levels as low as 0.09 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$ (Suter-Hafmann and Schlatter 1989). Prenatal exposure to $T_4CDD$ results in severe post natal immunosuppression by inhibiting thymocyte maturation (Holladay et al. 1991). Phenotypic changes in thymus observed perinatally are associated with persistent postnatal alteration immune function. Moreover, this alternation is achieved at dose levels that do not result in any other measurable development toxicity, such as litter size, number of resorptions, fetal body weight, and incidence of cleft palate (Holladay et al. 1991).

In mice, $T_4CDD$-induced thymus atrophy is genetically determined and mediated by the *Ah* receptor (Vos et al. 1997/98). The mode of action is unknown, although several scenarios have been ruled out. For example, studies indicate that the depletion of lymphocytes in the thymus is caused neither by a reduced production in thymic hormones nor a zinc deficiency. It is also unlikely that $T_4CDD$ requires metabolic activation. Thus, the action of $T_4CDD$ on the thymus is indirect (Vos et al. 1978). The effect of $T_4CDD$ on the thymus may be through an action on epithelium cells as high levels of the *Ah* receptor are found in thymic epithelium (Vos et al. 1997/98). Perinatal $T_4CDD$ exposure causes an alteration to the prothymocyte, resulting in reduced thymic seeding from both fetal liver and bone marrow during ontogeny. It appears that early steps in T-lymphopoiesis, before entry of stem cells into the thymus are especially sensitive to $T_4CDD$ exposure during the perinatal period (Fine et al. 1990; Lai et al. 1998).

In addition to thymus atrophy, suppression of the cellular immune response may be evidenced by reduced T-cell activity. For example, offspring of mice exposed to $T_4CDD$ (1.5 and 3.0 $mg \cdot kg^{-1}$ $bw \cdot d^{-1}$) during pregnancy (on gestation days 6 to 14) experienced a significant reduction in cytotoxic T-cell activity (Holladay et al. 1991). Similarly, Fine et al. (1990)

reported reduced fetal prothymocyte activities when pregnant mice were administered 15 $mg \cdot kg^{-1}$ bw on gestation day 14.

Captive harbour seals fed contaminated herring from the Baltic Sea for two years experienced impaired natural killer cell activity, *in vitro* T-lymphocyte function, antigen-specific *in vitro* lymphocyte proliferative responses, and *in vivo* delayed-type hypersensitivity and antibody responses to ovalbumin compared to those fed relatively clean herring from the Atlantic Ocean (Ross et al. 1996). Sister studies using the same herring batches on rats suggested that the increase in immunosensitivity may be due to an effect on the thymus. Moreover, perinatal exposure represents a greater immunotoxic threat than exposure as a juvenile or adult. The contribution of PCDD/Fs to the total TEQ profile diminished from herring to seals, suggesting that harbour seals may be able to preferentially metabolise the planar PCDDs (Ross et al. 1996).

In humoral immunity, lymphocytes that remain in the bone marrow develop into B-cells, which when mature, migrate to lymphatic tissue where they secrete antibodies. Because some B-cell antibody responses are regulated by T-cells, reduced host resistance to endotoxins such as *Streptococcus*, *Listeria*, *Salmonella*, and *Plasmodium*, may be caused by $T_4CDD$ effects on the thymus (Vos et al. 1991). Suppression of humoral immunity in adult mice, however, can occur at doses (1-5 $\mu g \cdot kg^{-1}$ bw) below those which cause thymic atrophy (5 $\mu g \cdot kg^{-1}$ bw), indicating that thymus is not directly involved (Tucker et al. 1986). For example, mice pre-treated with 1.5 $\mu g \cdot kg^{-1}$ bw of $T_4CDD$ once a week for four weeks experienced a slightly higher mortality rate (~20%) within two days of injection of $\geq 100 \cdot kg^{-1}$ bw of lipopolysaccharide (a T-cell independent endotoxin) than mice exposed solely to endotoxin. This dose of $T_4CDD$ is more than an order of magnitude lower than that which caused thymus atrophy at gross inspection (Vos et al. 1978). In thymus-independent cases, $T_4CDD$ may be inhibiting the differentiation, rather than proliferation, of B-cells to antibody-producing cells although several other possibilities may exist (Tucker et al. 1986; Vos et al. 1991).

Non-specific immunity, of which the complement system is a key component, is also sensitive to dioxin-induced suppression. A dose-dependent increase in susceptibility to *Streptococcus pneumoniae*, a bacterial pathogen whose host defence is complement mediated, was observed in mice exposed up to 10.0 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$ 1,2,3,6,7,8-HCDD for 14 d. Moreover, at doses of 10.0 $\mu g \cdot kg^{-1}$, suppression was maintained for 50 d after the last treatment; similar suppression was observed with treatment of 1.0 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$ of $T_4CDD$ (White et al. 1986).

Although the ecological relevance of immunological endpoints in often questioned, contaminant induced immunosuppression is gaining acceptance as a sensitive sub-acute indicator of adverse effects, including the potential for increased mortality (ATW 1998). For further information of the immunotoxic effects of $T_4CDD$, the reader is directed to a recent review by Vos et al. (1997/1998).

### 11.1.5 *Cancer*

Data from several studies indicate that chronic dietary exposure to low levels of $T_4CDD$ has the potential to result in an increased incidence of tumours in mammals (Table 38). Rats appear more sensitive than mice, and males more sensitive than females for both species. A working group of WHO convened to establish a TDI for humans concluded that $T_4CDD$ is carcinogenic in animals (but that results were inconclusive for humans) (cited in Schlatter 1994). It has also been argued that dioxin is a promoter blocker and a promoter, with a net effect of an anticarcinogen (Kayajanian 1997). This conclusion was based on one rat (Kociba et al. 1978) and two human exposure studies (Kayajanian 1997).

Kociba et al. (1978) established that $T_4CDD$ is a potent cancer promoter. In this study, male and female rats were fed diets containing 0, 0.022, 0.022, and 2.2 $\mu g$ $T_4CDD \cdot kg^{-1}$ for a period of two years. These dietary exposures were equivalent to 0, 0.001, 0.01, and 0.1 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$. Rats

fed 0.1 $\mu g \cdot kg^{-1} \cdot d^{-1}$ experienced increased mortality, increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue but reduced incidences of tumours of the pituitary, uterus, mammary glands, pancreas, and adrenal gland. Those fed 0.01 $\mu g \cdot kg^{-1} \cdot d^{-1}$ experienced a lesser degree of toxicity, but included liver and lung lesions. Rats fed 0.001 $\mu g \cdot kg^{-1} \cdot d^{-1}$ were comparable to the controls, though a significant increase in incidence of swollen hepatocytes was noted (Kociba et al. 1978). Recently, the histopathology slides of the hepatic lesions prepared in this study were re-evaluated using the current classification scheme used in the National Toxicology Program (NTP; Paustenbauch et al. 1991). While substantially fewer cancerous tumours were observed in this study using the new classification scheme, statistically significant increases in the incidence of hepatocellular adenomas were still evident in the 0.01 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$ treatment groups (Brown 1991; Keenan et al. 1991).

Male and female rats were administered doses of $T_4CDD$ by gavage, twice weekly, for a period of two years (U.S. Dept. of Health and Human Services 1982). Treatments corresponded to average daily doses of 0, 1.4, 7.1, and 71 $ng \cdot kg^{-1}$ $bw \cdot d^{-1}$. A dose-dependant increase in the incidence of thyroid tumours (follicular-cell adenomas or carcinomas) was observed in male rats, with the incidence in the high dose group significantly above that of the control group. In female rats, a similar but non-significant trend was evident. Female, but not male, rats demonstrated a dose-dependant increase in incidence of neoplastic nodules or hepatocellular carcinomas with a significant difference occurring at 71 ng $T_4CDD \cdot kg^{-1}$ $bw \cdot d^{-1}$. In an identical study in which male mice received the same doses as the rats, and female mice received 0.006, 0.03, and 0.3 $\mu g \cdot kg^{-1}$ $bw \cdot d^{-1}$, $T_4CDD$ was also carcinogenic in B6C3F1 mice, inducing heptocellular carcinomas in males and females and follicular-cell thyroid adenomas in females (U.S. Dept. of Health and Human Services 1982).

$H_6CDD$ doses of 0.036 and 0.071 $\mu g$ $TEQ \cdot kg^{-1}$ $bw \cdot d^{-1}$ were found to significantly increase the incidence of hepatocellular carcinomas or neoplastic nodules in female rats; a dose-dependent,

but non-significant increase was noted for males. Both male and female mice displayed a dose-dependant significant increase in incidence of heptocellular carcinomas or adenomas with significant effects occurring at the highest dose, 0.71 μg TEQ·kg$^{-1}$ bw·d$^{-1}$ for both sexes (U.S. Dept. of Health and Human Services 1980). The relative contributions of the two $H_6CDD$ isomers (1,2,3,6,7,8- and 1,2,3,7,8,9-HCDD) to the carcinogenic effects is not known.

## 11.2 Toxicity to Birds

Although the available data are limited, it appears that birds are slightly less sensitive than mammals to the effects of PCDD/Fs. Nonetheless, the effects associated with acute and chronic exposures to PCDD/Fs are similar, including lethality, reduced growth rates, liver enlargement, and reproductive impairment.

### 11.2.1 *Acute*

Only limited data exist on the acute oral toxicity of $T_4CDD$ to avian species but indicate that birds exhibit a broad range of sensitivities to this substance (Table 39). Single lethal doses ($LD_{50}$) of $T_4CDD$ range from 15 to > 810 μg·kg$^{-1}$ bw for bobwhite quail (*Colinus virginianus*) and ringed turtle dove (*Streptopelia risoria*), respectively (Hudson et al. 1984). Mallard ducks (*Anas platyrhynchos*) are also relatively resistant to this substance, with an $LD_{50}$ of > 108 μg·kg$^{-1}$ bw (Hudson et al. 1984). Leghorn chickens (*Gallus domesticus*) exposed to a single dose of 25-50 μg $T_4CDD$·kg$^{-1}$ bw died 12-21 days later with some birds experiencing weight loss and pericardial edema (Greig et al. 1973). In ring-necked pheasants (*Phasianus colchicus*), injection of 25 μg·kg$^{-1}$ bw resulted in reduced survival and growth in adult female birds (Nosek et al. 1992). Dose-dependent increases in mortality and decreases in body weight were observed in female ring-necked pheasants treated with a single doses of 0 to 100 μg $T_4CDD$·kg$^{-1}$ bw (Nosek et al. 1992).

### 11.2.2 *Chronic*

Few data were available to assess the effects of chronic exposures to $T_4CDD$ in birds, although data suggest that effects are similar to those observed in mammals (Tables 40 and 35, respectively). Significant mortality (80%) and edema occurred in juvenile leghorn chickens administered 1 μg $T_4CDD \cdot kg^{-1}$ bw$\cdot d^{-1}$ for a period of 21 d; at daily doses of 10 μg$\cdot kg^{-1}$ bw, 100% mortality occurred within 15 days. The NOEL for survival or edema in this study was 0.1 μg$\cdot kg^{-1}$ bw$\cdot d^{-1}$ (Schwetz et al. 1973). In a similar study, McKinney et al. (1976) reported significant effects on food consumption and body weight (i.e., reduced growth rate) when leghorn chickens were exposed to 1.0 μg TEQ$\cdot kg^{-1}$ bw$\cdot day^{-1}$ of $T_4CDF$ for 21 d; mortality was relatively high (16%) compared to control birds (0%). These effects are comparable to those reported in ring-necked pheasants administered an weekly doses of 1 μg $T_4CDD \cdot kg^{-1}$ bw (~0.14 μg $T_4CDD \cdot kg^{-1}$ bw$\cdot d^{-1}$) for 7 wk by ip injection (Nosek et al. 1992).

### 11.2.3 *Reproductive/Developmental*

Only one study was found that examined the effects of maternally administered dioxin on reproduction in birds (Table 41). Female ring-necked pheasants treated with weekly doses of 1 μg $T_4CDD \cdot kg^{-1}$ bw for 7 wk (~0.14 μg $T_4CDD \cdot kg^{-1}$ bw$\cdot d^{-1}$) experienced delayed onset of mortality in 57% of birds, significant reduction in egg production, and embryos from those eggs had a significantly higher cumulative percent mortality. There were no significant effects on fertility or eggshell thickness index (Nosek et al. 1992). Birds treated similarly with 0 to 0.1 μg $T_4CDD \cdot kg^{-1}$ bw (~0.014 μg $T_4CDD \cdot kg^{-1}$ bw$\cdot d^{-1}$) bw experienced no significant adverse effects.

Other studies directly injected eggs with known amounts of dioxin and examined the effects on developing chick embryos. Cheung et al. (1981) observed a significant dose-response relationship between $T_4CCD$ and incidence of cardiovascular malformations (up to 80%) in leghorn embryos 14 days after eggs were injected with doses of 0 to 0.453 $\mu g \cdot kg^{-1}$ egg; no such relationship was evident with mortality. Similarly, a dose dependant increase in overall embryo abnormality rate was observed when fertile white leghorn chicken eggs were injected once with up to 1 $\mu g \cdot kg^{-1}$ egg. Abnormalities seen here included: asymmetrical somites and heart abnormalities; discrepancies in developmental indicators; high frequency of abnormal visceral arches; underdeveloped brain and allantois; and missing tailbud (Henshel et al. 1993). These findings are consistent with the earlier conclusion of Verret (1970) who reported that as little as 0.01-0.02 $\mu g \cdot kg^{-1}$ egg of $T_4CDD$ in chicken eggs can produce embryotoxicity, edema and deformities (cited in Kubiak et al. 1989). It appears egg weight alone is not a sensitive indicator of toxic effects. Although concentration of $T_4CDD$ injected (0 to 1 $\mu g \cdot kg^{-1}$) into the yolk or air-cell contributed significantly, the most important factor affecting weight of 21 d chicken embryos was original weight of the egg (Henshel et al. 1997).

The effects of organochlorine contaminants on fish-eating bird populations has been extensively studied in the Great Lakes region and on the western Canadian coast. Results from studies employing field collected eggs demonstrate a strong association between exposure of dioxin-like compounds and impaired reproduction, although, the relative contributions to the toxic effects by individual compounds may very significantly between species and locations (Bosveld and van den Berg 1994). The relatively recent appearance of symptoms of PCDD/F toxicity may be due to declines in other organochlorine compounds, most notably DDT and its metabolite DDE. Owing to pesticide contamination, eggs were previously unable to survive long enough for PCDD/F toxicity to manifest (Giesy et al. 1994). Moreover, embryonic effects typically associated in dioxin toxicity were not routinely monitored until the 1980s. Nevertheless, dioxin toxicity itself has clearly lessened in recent years which correlates well with reductions in PCDD/F levels.

Unlike the Great Lakes, where PCBs contribute most of the TEQ activity (>95%), the Strait of Georgia in BC contains a relatively high proportion of PCDD/Fs making it an ideal location to study the effects of these compounds (Henshel et al. 1995). Great Blue Heron chicks hatched from eggs collected from five sites in and around the Strait of Georgia had asymmetric brains. Forebrain asymmetry correlated best with $T_4$CDD concentration in the egg, although forebrain depth was more strongly associated with TEQs (based on Safe 1990 TEFs), indicating that other PCDD/Fs may also influence brain development. Moreover, frequency and degree of asymmetry were notably higher in 1988 than 1990-92, when levels of $T_4$CDD and TEQ (based on Safe 1990 TEFs) concentrations had declined. $T_4$CDD and TEQ concentrations in the eggs ranged from non-detectable to 8.81 $ng \cdot kg^{-1}$ and non-detectable to 14.63 $ng \cdot kg^{-1}$, respectively (Henshel et al. 1995). Asymmetry was commonly associated with $T_4$CDD levels above 60 $ng \cdot kg^{-1}$, though an effect was observed at a concentration as low as 13 $ng \cdot kg^{-1}$ (Henshel 1998). $EC_{50}$s for asymmetry in brain angle, depth, height, and width are 53, 44, 40, and 32 ng $T_4CDD \cdot kg^{-1}$ and 99, 78.5, 83, and 64.5 ng $TEQ \cdot kg^{-1}$ (Safe 1990 TEFs), respectively (Henshel et al. 1998).

Chick edema disease, characterised by jelly-like subcutaneous edema on the breast, was observed in 33.3 and 15% of Great Blue Heron chicks hatched from eggs collected from Crofton and Vancouver, respectively; no edema was observed in chicks from Nicomekl (reference site) (Hart et al. 1991). Chicks from the Crofton site also measured significantly smaller in yolk-free weight, kidney and stomach weight, tibia length and weight, beak length, and down follicle density than those from Nicomekl; those from Vancouver had smaller kidney weights, tibia weight, and beak length. Levels of $T_4$CDD in the eggs were significantly correlated to: yolk-free weight, tibia length weight, beak length, and kidney and stomach weights. Mean $T_4$CDD levels in eggs were 211, 135, and 10 $ng \cdot kg^{-1}$ ww for the Crofton, Vancouver, and Nicomekl colonies, respectively (Hart et al. 1991). In a similar study, subcutaneous edema was diagnosed in Great Blue Heron chicks collected from Crofton BC in 1988 but not 1991. This observation corresponds to a significant decrease in PCDD and PCDF levels in heron eggs (both $T_4$CDD and

TEQ basis) at the site between the two years (from ~530 to 100 ng TEQ·kg$^{-1}$) (Sanderson et al. 1994a; based on Safe 1990 TEFs and include PCDD/Fs and PCBs). A similar trend was noted for herons collected at Vancouver from 1988 to 1992 (Sanderson et al. 1994a). Morphological measurements including, body weight, yolk weight, wing length, and brain asymmetry, were negatively correlated to TEQ levels (81 to 501 ng·kg$^{-1}$) in eggs of double-crested cormorants collected from five colonies across Canada (Sanderson et al. 1994a; Henshel et al. 1997).

Bald eagles appear to be relatively tolerant of dioxin toxicity. No significant concentration-related morphological, physiological, or histological effects were found in bald eagle chicks collected as eggs from pulp mill and references sites along the southern coast of BC (Elliott et al. 1996d). Total TEQ concentrations (WHO 1998 TEFs) ranged from 7596 ng·kg$^{-1}$ lipid in the egg yolks from West Vancouver Island to 25 627 ng·kg$^{-1}$ lipid in those from the Powell River (% lipid ~8.8-23%); PCDD/Fs accounted for 22-60% of the TEQ. The estimated NOEL and LOEL for hepatic CYP1A induction in bald eagle chicks are 100 and 210 ng TEQ·kg$^{-1}$ whole egg (WHO 1994 TEFs), respectively.

Similar studies have also been conducted in the United States and Europe. Reduced nest success, hatching success, survival, and weight, increased relative liver weight and incubation period were reported for Forster's terns from Lake Michigan (Green Bay, MI) for 1983 (Kubiak et al. 1989). $T_4$CDD, HCDD, total PCDD, and total PCDF concentrations at Green Bay were 37.3, 36.5, 101.5,and 18.5 ng·kg$^{-1}$egg, ww, respectively, while those of the reference site, Lake Pygan, were 8.0, 30., 25.0, and 9.0 ng·kg$^{-1}$egg, ww, respectively. Caspian terns (*Hydroprogne caspia*) may be less sensitive to dioxin activity as Ewins et al. (1994) found that overall, the conditions at Green Bay were relatively good for this species despite TEQ levels of approximately 500 ng·kg$^{-1}$egg, ww [based on WHO 1998 TEFs; includes PCDD/Fs (~8%)and PCBs (~92%)]. The authors concluded that the influence of contaminants, including PCDD/Fs, is now small relative to other stressors (e.g., human disturbances, predation).

Reproductive impairment was reported in wood ducks (*Aix sponsa*) collected downstream from a point source in Arkansas. Nests closest to the point source (9 km and 17 km downstream) experienced a significant reduction in eggs hatched and ducklings that left the nest compared to nests located 58 km and 111 km downstream (White and Seginak. 1994; White and Hoffman 1995). Of eggs that failed to hatch, 20% were cracked and desiccated; 45% were addled. All eggs were contaminated with a variety of PCDD/F congeners with average concentrations of individual congeners as high as 60 $ng \cdot kg^{-1}$ ww. Researches estimated a threshold TEQ level of 20-50 $ng \cdot kg^{-1}$, based on I-TEFs, for nest success, hatching success, and duckling production (White et al. 1994; White and Hoffman 1995).

Residue levels of PCDD/F TEQs (I-TEFs) in the yolk sac of cormorants (*Phalacrocorax carbo*) collected in The Netherlands were significantly correlated with head size, relative liver weight, shell weight, and yolk sac weight (van den Berg et al. 1994). Levels of individual congeners ranged from approximately 50 $ng \cdot kg^{-1}$ to 2.4 $\mu g \cdot kg^{-1}$ lipid. Common terns (*Sterna hirundo*) appear to be more sensitive as neither egg, hatchling, nor organ weights of those from The Netherlands were related to concentrations of dioxin-like compounds. The volume of eggs used for residue analysis were shown to be negatively correlated with TEQ concentrations in the yolk (Bosveld et al. 1995). The authors speculated that the absence of pronounced reproductive impairment in these birds compared to related species from the Great Lakes with comparable contaminant levels, was due to differences between PCDD/F and PCB congener profiles, particularly $T_4CDD$ (Bosveld et al. 1995). The NOEL from embryonic development in the common tern is below 4 $\mu g$ TEQ $\cdot kg^{-1}$ lipid (TEFs from **Bosveld et al. 1993**) (Bosveld and van den Berg 1994).

### 11.2.4 *Immune System*

Only a single study examining the effects of PCDD/Fs on the avian immune system was located

(Table 40). McKinney et al. (1976) reported marked thymic involution, reduced spleen weight and depletion of lymphocytes in the spleen of day old white leghorn chicks exposed to 1.0 μg TEQ·kg$^{-1}$ bw·day$^{-1}$ of $T_4$CDF for 21 d compared to control animals. Similar, but more pronounced, effects were observed in those dosed 5.0 μg TEQ·kg$^{-1}$ bw·day$^{-1}$.

### 11.2.5 *Cancer*

No studies were located on the carcinogenic effects of PCDD/Fs in avian species.

## 11.3 Toxicity to Amphibians and Reptiles

No toxicity studies were located on the effects of dietary consumption of PCDD/Fs to amphibians and reptiles. Water exposure and injection studies on amphibians discussed in Section 8.1.2 suggest that these species are relatively insensitive to the toxic effects of $T_4$CDD.

# 12. CANADIAN TISSUE RESIDUE GUIDELINE FOR THE PROTECTION OF WILDLIFE CONSUMERS OF AQUATIC BIOTA

For substances that are persistent and bioaccumulative, the main route of exposure for wildlife in aquatic ecosystems is the consumption of contaminated aquatic prey species such as fish. In order to address this route of exposure, tissue residue guidelines (TRGs), which are maximum concentrations of chemical substances in aquatic biota, are developed to protect, restore, and sustain wildlife that consume aquatic biota in freshwater, estuarine, and marine ecosystems. TRGs can apply to tissue residues in dietary species including fish, shellfish, invertebrates, or aquatic plants that are consumed by wildlife (e.g., piscivores, insectivores, and herbivores). In addition to other environmental quality guidelines, TRGs provide benchmarks to help interpret biological monitoring data and serve as the scientific basis for determining interim management objectives and performance indicators to measure progress in virtual elimination strategies.

## 12.1 CCME Protocol

To develop the dioxin/furan tissue residue guideline, laboratory studies involving chronic exposure of dietary PCDD/Fs to mammals and birds were thoroughly evaluated for their usefulness in guideline development. Studies were ranked as primary, secondary, or ancillary as outlined by CCME criteria (1991a; 1998). The ecological relevance of many studies involving slight effects, though statistically significant, is debatable. Thus, sensitive endpoints, such as embryonic development, early survival, growth, reproduction, and adult survival were preferred over behavioural, biochemical, or other endpoints (e.g., lesions, adult organ/body weight changes). Owing to limited data, preference could not be given to studies employing wildlife species. A few novel studies did not utilize pure congeners, but rather incorporated fish contaminated with known amounts of dioxin-like compounds into the diets of test animals (Heaton et al. 1995a, b; Ross et al. 1996; Tillitt et al. 1996, Arnold et al. 1998; Feeley and Jordon

1998; Feeley et al. 1998). Studies involving secondary toxicity were not considered for guideline derivation because of the presence of contaminants other than dioxins in the diet items. Nonetheless, these studies provide insight into overall toxicity of natural food sources and were considered as part of the weight-of-evidence.

For all studies, concentrations of PCDD/F congeners administered to test animals were converted to $T_4$CDD toxic equivalents using the most recent TEFs from WHO (van den Berg et al. 1998). Average daily doses of TEQs were calculated, if necessary, using the food intake:body weight ratios (FI:BW) provided in the original paper, the national TRG Protocol (CCME 1998), or other sources (e.g., U.S. EPA 1993). Tolerable daily intakes (TDIs) for PCDD/Fs in food were then determined, according to protocol.

$$TDI = (LOEL \cdot NOEL)^{0.5} \div UF$$

where;

TDI = Tolerable daily intake (ng TEQ·kg$^{-1}$ bw·d$^{-1}$);

LOEL = Lowest observed adverse effect level (ng TEQ·kg$^{-1}$ bw·d$^{-1}$);

NOEL = No observed adverse effect level (ng TEQ·kg$^{-1}$ bw·d$^{-1}$);

UF = Uncertainty or safety factor.

For some cases in the dioxin/furan data set, the LOEL was the lowest concentration tested, with the NOEL therefore laying somewhere between the control (normally 0 ng TEQ·kg$^{-1}$ bw·d$^{-1}$) and the LOEL. In these instances, the NOEL was estimated by a formula that was derived from animal toxicological studies with various pesticides (CCME 1993; 1998):

$$NOEL = LOEL \div 5.6$$

The value of 5.6 is the upper 95% confidence limit for a mean LOEL:NOEL ratio of 3.93.

Therefore, dividing the LOEL by 5.6 should safely estimate the NOEL in approximately 95% of the cases (CCME 1993). There is no evidence to suggest that this relationship does not hold for dioxin and furan toxicity data. From the data set assembled here, the mean LOEL:NOEL ratio and its corresponding 95% confidence interval for mammalian species (n=12) are 5.1 and 3.34-5.82, respectively. There was insufficient data available to calculate LOEL:NOEL ratios for avian species.

In general, uncertainty factors (UF) are used to account for uncertainty in the estimate of the TDI due to limitations in the toxicological data (CCME 1998). More specifically, uncertainty factors are applied because the toxicological database may be insufficient to fully evaluate differences in sensitivities to toxicants arising from gender, life stage, species tested, duration of exposure, endpoint measured, and exposure route.

The lowest mammalian and avian TDIs derived from chronic studies were then used in conjunction with the body weights (BW) and daily food intake rates (FI) of wildlife species to calculate reference concentrations (RCs) of PCDD/F TEQs, using the following equation. For the purposes of deriving a national value, the mammalian and avian RCs must be as inclusive as possible to accommodate all species and regions in Canada and, therefore, are based on the highest mammalian and avian FI:BW known for Canadian wildlife, namely 0.24 for female mink and 0.94 for Wilson's storm petrel (CCME 1998). These RC values should be applied to the highest trophic level at which the given wildlife species feeds.

$$RC = TDI \cdot (BW \div FI)$$

where;

RC = Reference concentration (ng TEQ·kg$^{-1}$ diet on a wet weight basis);

TDI = Tolerable daily intake (ng TEQ·kg$^{-1}$ bw·d$^{-1}$);

BW = Body weight (kg ww); and

FI = Food intake rate (kg ww·d$^{-1}$).

We recognise that use of the highest FI:BW ratio may not always be appropriate (e.g., in areas where Wilson's storm petrel is not found). For this reason, a list of RCs for a suite of mammalian and avian receptors is provided (see Table 42).

### 12.1.1 *Reference Concentrations (RCs) for the diets of mammalian species*

Tolerable daily intakes as low as 0.04 ng TEQ·kg$^{-1}$ bw·d$^{-1}$ were calculated from mammalian data, but as these studies examined subtle effects (e.g., enzyme induction; swollen hepatocytes) in rats, their ecological relevance is questionable and therefore not considered directly for guideline derivation purposes (Tables 33 and 38; Birnbaum et al. 1989b; Kociba et al. 1978). Furthermore, although a TDI of 0.04 ng TEQ·kg$^{-1}$ bw·d$^{-1}$ was also calculated for reduced pup survival in rats, pup survival increased relative to controls when parents were mated a second time (Murray et al. 1979). Instead, a TDI of 0.17 ng TEQ·kg$^{-1}$ bw·d$^{-1}$ for significantly reduced growth rates in male and female weanling guinea pigs (De Caprio et al. 1986) was selected as the starting point for deriving mammalian RCs. Reduced growth of young is considered an ecologically significant endpoint (CCME 1998). In this study weanling guinea pigs were fed diets containing 0 to 26 ng $T_4$CDD·kg$^{-1}$ diet for 90 d. There were no observable effects at 0.1 or 0.6 ng $T_4$CDD·kg$^{-1}$ diet. At 4.9 ng $T_4$CDD·kg$^{-1}$ diet, male and female guinea pigs experienced a 39 and 22% reduction in growth rates, respectively, compared to controls. At 26 ng $T_4$CDD·kg$^{-1}$ diet, guinea pigs lost weight, and 60% died or had to be sacrificed (De Caprio et al. 1986). On a TEQ basis, the NOEL and LOEL are 0.6 and 4.9 ng TEQ·kg$^{-1}$ diet, respectively, as the TEF value for $T_4$CDD is one (van den Berg et al. 1998). The geometric mean of the LOEL and the NOEL divided by an uncertainty factor of ten gives a TDI of 0.17 ng TEQ·kg$^{-1}$ bw·d$^{-1}$. This study was chosen because: (i) exposure was relatively long-term (90 d); (ii) it reported an adverse effect on an ecologically significant endpoint (growth of

young); (iii) several dose levels were tested, with a clear dose-response effect; and (iv) $T_4CDD$ concentrations in the diet were measured.

Toxicity data exist for several other sub-chronic and chronic studies in which various strains, life stages, and genders of mammalian species were tested. In addition, a number of sensitive end-points, such as growth and reproduction, are reflected in the data base. Toxicity data on sensitive aquatic predators (e.g., mink) is limited, however. As such, an uncertainty factor of ten was chosen to adjust from a sub-chronic to chronic and to accommodate differences in interspecies sensitivities to PCDD/Fs.

A RC of 0.71 ng TEQ·kg$^{-1}$ diet was obtained by dividing the TDI for guinea pigs (0.17 ng TEQ·kg$^{-1}$ bw·d$^{-1}$) by the highest FI:BW ratio for wild mammals (0.24 for female mink) (Table 42; CCME 1998).

### 12.1.2 *Reference Concentrations (RCs) for the diets of avian species*

Only limited information was available on the effects of orally administered PCDD/Fs to birds. These data were sufficient, however, to meet the minimum data requirements for an interim guideline (CCME 1998). In a 21 d test, survival, food consumption, and body weights were reduced in one day old white leghorn chicks dosed 1 µg TEQ·kg$^{-1}$ bw·d$^{-1}$ by oral intubation (2,3,7,8-TCDF) (McKinney et al. 1976). Similar, but more pronounced effects, including 100% mortality, were observed in those dosed 5.0 µg TEQ·kg$^{-1}$ bw·day$^{-1}$ (McKinney et al. 1976). A NOEL of 0.18 µg TEQ·kg$^{-1}$ bw·d$^{-1}$ was calculated by dividing the LOEL of 1 µg TEQ·kg$^{-1}$ bw·d$^{-1}$ by 5.6 because this LOEL was also the lowest dose tested (CCME 1998). A TDI of 42.4 ng TEQ·kg$^{-1}$ bw·d$^{-1}$ for white leghorn chickens was derived by dividing the geometric mean of the LOEL and NOEL by ten. For ring-necked pheasants, a NOEL and LOEL of 0.014 and 0.14 µg TEQ·kg$^{-1}$ bw·d$^{-1}$, respectively, for significantly reduced egg production and increase in

mortality of embryos were calculated (Nosek et al. 1992). In this study, ring-necked pheasants were dosed via ip injection once a week with 0, 0.01, 0.1, or 1.0 $\mu g \cdot kg^{-1}$ for seven weeks (Nosek et al. 1992). A TDI 4.47 ng $TEQ \cdot kg^{-1}$ $bw \cdot day^{-1}$ for ring-necked pheasants was derived by dividing the geometric mean of the LOEL and NOEL by an uncertainty factor of ten.

Dietary toxicity tests for PCDD/Fs on wild and domestic avian species is very limited; only two sub-chronic studies were found, both of which are ranked secondary. The chicken study (McKinney et al. 1976) was ranked secondary because no statistics were performed and the ring-neck pheasants study (Nosek et al. 1992) was ranked secondary because dosing occurred via intraperitoneal injection once a week (CCME 1998). For both studies, an uncertainty factor of ten was chosen to adjust from a sub-chronic to chronic study and to accommodate differences in interspecies sensitivities to PCDD/Fs and exposure routes.

That the TDI (42.4 ng $TEQ \cdot kg^{-1}$ $bw \cdot d^{-1}$) for white leghorn chickens is higher than that for ring-necked pheasants (4.47 ng $TEQ \cdot kg^{-1}$ $bw \cdot day^{-1}$) is inconsistent with reports indicating that white leghorn chickens may be inherently ten times more sensitive to $T_4CDD$ and $T_4CDF$ exposure than ring-necked pheasants based on EROD inducing potency (Kennedy et al. 1996b). A probable explanation for this discrepancy is the use of different exposure routes (intubation vs. injection) and different sensitivities among measured endpoints (growth vs. egg production and embryo mortality).

Dividing the lowest TDI (4.47 ng $TEQ \cdot kg^{-1}$ $bw \cdot d^{-1}$) by the highest FI:BW for wild birds (0.94 for Wilson's storm petrel) results in an avian RC of 4.75 ng $TEQ \cdot kg^{-1}$ diet (Table 42).

## 12.2 Tissue Residue Guidelines from other Jurisdictions

Tissue residue guidelines have been developed both for the protection of human health and the

protection of wildlife consumers of aquatic organisms. In Canada, Health and Welfare Canada (HWC 1990) has derived a guideline of 20 ng TEQ·kg$^{-1}$ in fish and shellfish for the protection of human health. A similar level (15 ng TEQ·kg$^{-1}$) is currently being used in Ontario to evaluate the suitability of sportsfish tissues for human consumption (OMOE 1992). Previously, the IJC had recommended a guideline of 10 ng·kg$^{-1}$ for $T_4CDD$ in the tissues of aquatic organisms (Boddington et al. 1990). More recently, Quebec has recommended criteria of 0.07 and 0.66 ng·kg$^{-1}$ $T_4CDD$ in aquatic life tissue for the protection of human health and piscivorous wildlife, respectively (MEF 1998).

The U.S. EPA (1993) has reported low risk levels of $T_4CDD$ in fish tissues for the protection of various components of the aquatic ecosystem. For the protection of fish, the low risk concentration was 50 ng·kg$^{-1}$. In comparison, the low risk levels for mammalian and avian wildlife species were 0.7 and 6.0 ng·kg$^{-1}$, respectively. The U.S. EPA (1995) also proposed a Great Lakes water-based criteria of 0.0031 pg·L$^{-1}$ to protect wildlife from the effects of $T_4CDD$ that has bioaccumulated in the tissues of aquatic biota. In New York, the recommended fish tissue criterion for the protection of wildlife species from the potential carcinogenic effects of $T_4CDD$ is 2.3 ng·kg$^{-1}$. A slightly higher value of 3.0 ng·kg$^{-1}$ was proposed when only non-carcinogenic effects of $T_4CDD$ were considered (Newell et al. 1987).

Environmental quality objectives for PCB-based TEQs for otter and fish have been developed in The Netherlands using correlations between PCB TEQ and hepatic retinoid (vitamin A) concentrations (Smit et al. 1996). In otters, a TEQ concentration of 2 µg·kg$^{-1}$ is deemed a safe level (=$EC_1$) while 5 µg·kg$^{-1}$ is considered critical (=$EC_{90}$). Safe (=$EC_1$) and critical (=$EC_{90}$) TEQ levels in fish, obtained by dividing the TEQ levels in otters by the BMF, are 0.7 and 1.8 ng·kg$^{-1}$ ww (or 11 and 29 ng·kg$^{-1}$ lipid) in the total otter diet, respectively (Smit et al. 1996).

## 12.3 Canadian Tissue Residue Guideline

The lowest RC between the mammalian and avian values, 0.71 ng TEQ·kg$^{-1}$ diet, is adopted as the dioxin/furan TRG (Table 42). The guideline refers to the TEQ concentration due to PCDD/Fs measured in an aquatic organism on a wet weight basis that is not expected to result in adverse effects on wildlife in freshwater, marine, and estuarine systems. This guideline is considered interim as avian toxicity data was only sufficient to satisfy minimum requirements for an interim guideline. And, as no dietary toxicity data were located for amphibian and reptilian species, this interim guideline applies only to mammalian and avian wildlife.

### 12.3.1 *Data Gaps*

To upgrade the Canadian TRG for dioxins and furans from interim to full guideline status, at least two primary toxicity studies on two avian species are required. At least one of these studies must be a subchronic or chronic test considering sensitive endpoints (e.g., reproduction, development, growth, or survival of young). Studies on traditional avian laboratory or domestic species (e.g., chicken) may be used; however, studies on wildlife species that feed on aquatic organisms are preferred (CCME 1998). Moreover, treatment through dietary ingestion is preferred over other methods (e.g., injection, intravenous, dermal). The toxicity data that are currently available on mammals is generally sufficient for evaluating the toxic effects of $T_4CDD$, although limited data (three studies) were available for mink, presumed to be one of the most sensitive mammalian wildlife species. For both mammalian and avian species, little information was located on the toxicity of individual congeners other than $T_4CDD$. Few studies were located on the effects of PCDD/F mixtures and more complex mixtures containing other contaminants (e.g., PCBs). Non-additive effects and interactions among dioxin-like and non-dioxin-like congeners need to be further elucidated (Safe 1998). Additionally, there is a paucity of information regarding the bioaccumulation and/or biomagnification of PCDD/Fs in wildlife that

prey on aquatic biota. A greater understanding of the processes involved in uptake, elimination, metabolism, and biotransformation of PCDD/Fs as their levels in some organisms are very low. This information would better establish the relative risks of PCDD/Fs in the environment.

## 12.4 The Canadian TRGs for PCDD/Fs and PCBs

### 12.4.1 *Comparisons among Mammalian and Avian RCs for PCDD/Fs and PCBs*

Dioxins, furans and non-*ortho* and mono-*ortho* PCBs share a similar mode of action, but induce varying degrees of toxic responses. It is generally accepted in toxicology that a relatively weak inducer, but one that is abundant in a system, has the same effect as a strong inducer that is present in only small amounts. By applying TEF values to the concentrations of individual PCDD, PCDF, and PCB congeners, one can theoretically correct for the differences in toxicities among the congeners, thereby facilitating comparisons among systems with different chemical profiles. As such, one would expect that the TEQ guidelines derived for dioxins and furans, and PCBs would be similar in magnitude, regardless of the nature of the chemicals used in the derivation.

The mammalian RCs for PCDD/Fs (0.71 ng TEQ·kg$^{-1}$ diet) and PCBs (0.79 ng TEQ·kg$^{-1}$ diet) are remarkably similar (see also Environment Canada 1998b). This TEQ threshold is further supported by the low risk $T_4$CDD level of 0.7 ng·kg$^{-1}$ proposed for mammalian wildlife species by the U.S. EPA (1993) and by the PCB TEQ criteria of 0.7 ng TEQ·kg$^{-1}$ diet developed by The Netherlands to protect the otter (Smit et al. 1996). Strong support also comes from a study in which mink were fed fish contaminated with a suite of PCDD, PCDF, and PCB congeners throughout the reproductive period (Heaton et al 1995a, b; Tillitt et al. 1996). The TDI calculated for total TEQs (PCDD/Fs and PCBs) from this study was 0.2 ng TEQ·kg$^{-1}$ bw·d$^{-1}$, and if divided by the FI:BW ratio for female mink (0.24), would give a concentration in the diet of

0.8 ng TEQ·kg$^{-1}$. In this study, the researchers found that a concentration of 22 ng TEQ·kg$^{-1}$ in the fish significantly reduced kit growth.

The fact that mammalian RCs are ten-fold smaller than avian RCs suggests that avian species are less sensitive to the effects of dioxin-like compounds than mammals. The RC developed from avian PCB data (2.4 ng TEQ·kg$^{-1}$ diet; Environment Canada 1998b) is approximately half of the RC derived from PCDD/F toxicity data (4.75 ng TEQ·kg$^{-1}$ diet) presented here. Considering that the PCDD/F data set for avian species was very limited, that much of the PCB dietary toxicity testing was completed prior to the development of sensitive analytical techniques, that many of the TEQ dietary intakes had to be estimated using nominal concentrations in the food and body weights and food consumption rates from in unrelated studies, PCDD/F and PCB avian RCs are in good agreement with each other. The difference may be an indication that TEFs can be further refined, or that species specific TEFs may be required in some cases. Further, variability exists in PCB congener concentrations for the same technical mixture. Thus, the TEQ conversion factors used to convert commercial PCB mixture concentrations to a TEQ basis may over or underestimate the true TEQ content for a given study.

## 12.5 Guideline Implementation Considerations

### 12.5.1 *Concurrent use of the PCDD/F and PCB TRGs*

The Canadian Tissue Residue Guidelines for PCDD/Fs and PCBs can not be considered in isolation. In environments where PCDD/Fs are known to clearly dominate on a TEQ basis in aquatic organisms, the PCDD/F TRG will protect wildlife consumers of aquatic biota. Where a principal aquatic prey item contains both PCDD/F and PCB levels at or below their respective guideline values, consumers may not be protected against adverse effects because of the chemicals' common mode of action. In these environments where both PCDD/Fs and PCBs contribute significantly to the TEQ concentration, the lower PCDD/F TRG should take

precedence as the total TEQ concentration (i.e., PCDD/F and PCB TEQs combined) not to be exceeded.

Site-specific PCDD/F and PCB guidelines may be calculated based on their respective relative contributions to TEQ levels within target organisms. For example, if an important prey item of a mammalian species has a TEQ level in excess of the PCDD/F TRG, and that PCDD/Fs are known to contribute 30% to the total TEQ, than the site-specific PCDD/F TRG objective could be, for example, 0.21 $ng \cdot kg^{-1}$ (i.e., $0.3 \cdot 0.71$ $ng \cdot kg^{-1}$).

### 12.5.2 *Monitoring total TEQ levels*

As bioassays take on a greater role in toxicology, they could become an important part of routine contaminant analysis (see Section 7.3). For example, a bioassay could be used as a quick and inexpensive primary tool with which to screen for the presence of significant quantities of dioxins and dioxin-like compounds. Should results of such a test fall below the PCDD/F TRG, then further testing may not be required. If the results exceed the recommended value, then chemical analysis could be completed in a step-wise fashion, depending in part, on the contaminant history of the location.

### 12.5.3 *Trophic level considerations*

For substances that have a strong potential to biomagnify through the food chain, proper implementation of the tissue residue guidelines (TRGs) will require consideration of trophic level specific factors. Most important factors include the identification of the wildlife species requiring protection, the food preferences of those wildlife species, and the trophic level at which the species of concern feeds. To protect all wildlife at a site, the TRGs should be applied to the highest known aquatic trophic level.

The following sections and accompanying tables (Tables 43 to 48) are intended to provide general guidance necessary for the proper implementation of TRGs for bioaccumulative substances. The classification for aquatic trophic levels (Tables 43 to 45; from U.S. EPA 1995) provides guidance on the aquatic organisms found at each trophic level, as well as the wildlife species that feed at each aquatic trophic level in both freshwater and marine environments. Feeding habits for several consumers of aquatic life are presented (Tables 46 and 47) to aid guideline users in choosing an appropriate trophic level at which to apply the guideline to protect the wildlife species of concern. Use of food chain multipliers (Table 48; from Sample et al. 1996) is only one approach of many that may be suitable for estimating trophic-level specific chemical concentrations.

#### 12.5.3.1 Uncertainties with Establishing Distinct Trophic Levels

A major difficulty with the trophic levels described above is that many species feed at more than one trophic level and therefore do not fit wholly into discrete trophic levels. While species can be fairly readily identified as plants or herbivores, the carnivorous species are difficult to categorize into distinct trophic levels. For example, lake trout will consume both benthic invertebrates (trophic level 2) and small fish (trophic level 3). Mink will feed on muskrats (which feed mainly on emergent vegetation, therefore trophic level 1), shiners (trophic level 3), and some walleye (trophic level 4). In general, the closer a species is to the top of a food web, the more likely it is to feed on prey from more than one trophic level (U.S. EPA 1995). To overcome the uncertainty in assigning the diet of a wildlife species to a specific trophic level, the highest trophic level at which a species feeds was used for its classification.

Uncertainty also exists when attempting to estimate the food chain or web that supports a given wildlife species in a specific location. First, a food chain analysis implies a certain consistency

in feeding patterns over time. The diets of animals, however, vary both with season because of changes in nutritional needs and availability of prey, and with organism age and size as larger animals can take larger prey. Thus, analysis of a particular food chain or web for an organism over a short period of time may not be indicative of the potential for bioaccumulation and biomagnification over longer time periods.

Finally, ecosystem types that would otherwise be similar (e.g., oligotrophic lakes of a certain size) may support substantially different food webs and numbers of trophic levels to the top predators depending on the history of the ecosystem, species introductions, and species loss. It has been shown that much of the large between-lake differences in contaminant levels among fish species results from differences in the length of the food chains (Rasmussen et al. 1990). This underlines the importance of understanding local food webs if the TRGs are being applied on a site-specific basis.

#### 12.5.3.2 Basic Aquatic Trophic Levels

General trophic level guidance for freshwater and marine (salt marsh and open water) ecosystems is provided in Tables 43, 44, and 45. Each trophic level has a general description of the species that feed within that trophic level and what their diet resembles. These tables are not intended to replace best scientific judgement at a given site.

#### 12.5.3.3 Prey Trophic Levels of Representative Species

Information on the feeding habits and prey trophic levels of several species in both freshwater and marine ecosystems is presented in Tables 46 and 47. Again, these tables are only meant for guidance and are not intended to replace best scientific judgement at a given site.

#### 12.5.3.4 Food Chain Multipliers

There may be occasions where contaminant data does not exist for the aquatic trophic level at which the species of concern feeds. In this case, the use of food chain multipliers will allow the investigator to estimate what the concentration of the substance will be in other trophic levels. A list of aquatic food chain multipliers is presented in Table 48.

Food chain multipliers are specific for the log $K_{ow}$ of a particular substance. For example, if mink (which feeds at trophic level 3) were a species of concern at a site contaminated with $T_4CDD$, and data only existed for $T_4CDD$ concentrations in trophic level 2 zooplankton, then food chain multipliers could be used to predict the concentration of $T_4CDD$ in trophic level 3 fish. The mean log $K_{ow}$ for $T_4CDD$ is ~6.9 (Table 5). Therefore, according to Table 48, the concentration of $T_4CDD$ in zooplankton would be multiplied by 14.388 to estimate the concentration of $T_4CDD$ in trophic level 3 fish. This and other PCDD/F congener concentrations adjusted for trophic level could then be converted to a TEQ and summed to give estimated TEQ for trophic level 3 fish. This TEQ value would then compared to the PCDD/F TRG to determine if mink at that site are at risk from PCDD/F contamination. Food chain multipliers may be also used to estimate chemical concentrations of lower trophic level organisms by dividing the concentration of higher trophic level species.

## 12.6 Future Directions for Canadian TRGs

### 12.6.1 *Guideline Derivation*

Given that PCDD/Fs and coplanar PCBs share a similar mode of action, and that their relative toxicities can now be standardized through the use of TEFs, a single guideline developed from a

combined PCDD/F-PCB database would be most ecologically relevant. Further, other chemicals such as PAHs may prove to act in a similar fashion, and thus lend support to a total TEQ guideline for the combined effects of all *Ah* receptor active chemicals, rather than individual guidelines based on concentration alone. Prior to developing such a guideline, it will have to be confirmed that *Ah* receptor mediated toxicity is the most sensitive response sparked by these compounds (i.e., confirm that such substances do not have a second, more potent mode of action).

With respect to guideline development for the protection of avian consumers of aquatic life, a guideline based on TEQ concentration in the egg may be more ecologically relevant and easier to implement than the current Tissue Residue Guidelines based on dietary intake. Currently, several seabird, raptor and herring gull monitoring programs track contaminant levels in the eggs of these birds. Eggs are easier to collect and analyze, and contaminant levels in the eggs are a better predictor of potential reproductive effects than those found in adult birds. Further, the diets of these birds are often so varied that it would be difficult to decide which aquatic prey species would be the best indicator of the birds' health. Research for this type of guideline is growing rapidly; for example, many recent studies have examined the reproductive impacts by injecting fertilized eggs directly with $T_4CDD$ or, one or more PCB congeners rather than orally dosing adult birds (Brunström 1988; Powell et al. 1996a, b).

### 12.6.2 *Guideline Implementation*

The Canadian Wildlife Service is developing a computerized model entitled "Wildlife Contaminant Exposure Model (WCEM)" that will provide data and guidance on selection of trophic level specific factors. Currently, WCEM contains information on weight, diet, and intake rates for respective avian and mammalian species in addition to some chemical specific data. The data base will be updated in the near future to provide also trophic level and life history

information (L. Brownlee, CWS, pers. comm. 1998). One of the program's objectives will be to derive site and trophic level specific Canadian TRGs.

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