Semi-aquatic wildlife species have been important sentinels of environmental health and remain important biomonitors concerning emerging contaminant issues. The semi-aquatic wildlife of the Laurentian Great Lakes (LGL) has led the way in this regard and continues to do so. This is because the LGL and associated land mass possess a set of unique characteristics that make it an ideal laboratory for identifying the presence of environmental contaminants and their effects on semi-aquatic wildlife. The LGL basin and its diverse habitats support a wide array of wildlife with a significant number of species being dependent on the preponderance of coastline habitats. These species living in the transition areas of aquatic and terrestrial environments are exposed to contaminants from broad sources. As a result, semi-aquatic wildlife species often experience exposure to contaminants that are greater than for either terrestrial- or aquatic-based species and thus have been key sentinels of environmental health, both in the LGL basin and elsewhere. For the LGL, human activities including industrial, agricultural and recreational have resulted in significant inputs of anthropogenic contaminants. The combination of contaminant input and shoreline habitats with slow water turnover rate results in a great potential for chronic contaminant exposure of semi-aquatic wildlife species. In 1962, environmental awareness was brought to the forefront when the observations of a LGL scientist were published in Rachel Carlson’s *Silent Spring*. The assumptions that environmental contaminants were not very toxic and that the dilution potential of the LGL was infinite were clearly in error. Over the next 30 years, declines in individual and population health of LGL semi-aquatic species including mink, otter, bald eagles, terns, cormorants and numerous amphibian species warned the world of the potential for widespread, adverse impacts of contaminants on the environment. Compounds such as DDT, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and a great number of pesticides were being identified in the tissues of these species at concentrations associated with adverse effects. Today, through the combined work of scientists, government and industry, exposure to identified contaminants has been reduced within the LGL. As a result, populations of mink, eagles and cormorants and other semi-aquatic wildlife species have seen increases over the last two decades and the lessons learned have pushed this trend worldwide. Today more than 600 programmes are utilised to monitor the environmental health
of the eco-laboratory we call the LGL (www.glc.org/monitoring/greatlakes/). Semi-aquatic wildlife species continue to be a key component of that effort.

### Laurentian Great Lakes

Lakes Erie, Huron, Michigan, Ontario and Superior comprise the Laurentian Great Lakes, which together form the world’s largest fresh water system. The Great Lakes have a total volume of 23,000 km$^3$, a surface area of 244,000 km$^2$ and a drainage area of 750,000 km$^2$. The Great Lakes represent 80% of the total surface water of North America and 20% of the world’s freshwater supply (Li et al., 2006; Ward et al., 2008). These collective waters are a vital economic and environmental resource for both the United States and Canada. Economically, the area generates over $330 billion in US/Canada trade, accounting for 18% of the gross domestic product of the two countries (Zhu and Hites, 2004; Ward et al., 2008). The basin is rich in terrestrial and aquatic wildlife, including over 100 species that are considered to be rare, endangered or at risk.

Because of the economic benefits associated with being in close proximity to a natural resource of this magnitude, the area surrounding the Great Lakes is densely populated and highly industrialised. Forty million people, representing 10% of the United States population and 30% of the Canadian live within the Great Lakes basin. As a result of contaminant runoff from urban, agricultural and industrial sources and atmospheric deposition, in excess of 1,000 chemicals have been identified in the water column or resident biota. Approximately 350 of these chemicals are present in significant quantities (Ward et al., 2008).

### Impact of Contaminants on LGL Wildlife

In the mid-1960s, the impact of chemicals on the health of semi-aquatic wildlife species within the LGL began to concern biologists. Impaired health of individuals, reproductive failures and population declines of mink, eagles and a number of colonial fish-eating birds were noted. For avian species, the signs observed were consistent with those of ‘chick oedema disease’ in domestic chickens (*Gallus domesticus*), which included oedema, hydropericardium, ascites, liver enlargement, porphyria, hepatic necrosis with fatty degeneration and a high rate of mortality following exposure to PCBs (Vos and Koeman, 1970; Vos, 1972; Gilbertson, 1983). A similar relationship was established for PCB exposure and impaired reproductive performance of mink on commercial fur farms within the LGL basin (Aulerich et al., 1971). Later, Ludwig et al. (1996) determined that there was a relationship between exposure to PCBs and the structurally similar PCDDs and PCDFs and the increased incidence of embryonic deformities and mortalities in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) nesting in the LGL. Regional studies indicated decreased hatching success and increased incidence of nestling deformities in a colony of double-crested cormorants located in an area along Lake Michigan contaminated with PCBs when compared with a reference colony (Larson et al., 1996). Threshold concentrations of PCBs and PCB-like contaminants in eggs were suggested, based on a study examining the contaminant burden and the impaired reproductive success of LGL Forster’s terns (*Sterna forsteri*) (Kubiak et al., 1989). The severity of reproductive failure observed in colonies of herring gulls (*Larus argentatus*) throughout the LGL appeared to be directly related to contaminant concentrations in eggs, although a casual relationship could not be established (Gilbertson, 1983). While similar observations of impaired wildlife health were noted elsewhere, this suite of signs became collectively known as the Great Lakes embryo, mortality, oedema and deformities syndrome (GLEMEDS) (Gilbertson et al., 1991).

### Polychlorinated Contaminants

As suggested above, PCBs, PCDDs and PCDFs have been scrutinised for the past 40 years as potential contaminants responsible for a number of the effects observed in wildlife residing in the LGL basin. The PCDDs, PCDFs and PCBs are widely distributed into the global environment and can be very resistant to environmental degradation and me-
As a result, they readily accumulate in the food chain, with the greatest tissue concentrations being found in species at the higher trophic levels. Residues have been detected in a variety of animal species, including humans (Van den Berg et al., 1994; Safe, 1998). In some situations, the environmental concentrations of these contaminants are such that there are concerns about the health of wildlife and humans. Because of this concern, there continues to be an effort on the part of state, federal, and international regulatory agencies to minimise exposure to this significant class of environmental contaminants.

PCBs are formed by substituting chlorine for hydrogen on the biphenyl molecule, which consists of two benzene rings (Figure 16.1). Theoretically, there are 209 possible PCB congeners considering the five chlorine-binding sites on each ring. Each congener has been assigned a unique number from 1 to 209 in accordance with the rules of the International Union of Pure and Applied Chemistry (IUPAC). Commercial PCB products are mixtures of congeners that differ with respect to the extent and positions of chlorination. Polychlorinated dibenzo-\(p\)-dioxins are composed of two benzene rings connected by two oxygen atoms and contain four to eight chlorines, for a total of 75 congeners (Figure 16.1). Polychlorinated dibenzofurans are also composed of two benzene rings. The rings have a single oxygen between them and have four chlorine binding sites available on each ring (Figure 16.1). There are 135 different PCDF congeners (Di Carlo et al., 1978; Safe, 1990, 1998; Headrick et al., 1999; Huwe, 2002; Mandal, 2005; Schecter et al., 2006).

Certain approximate stereoisomers in this group, often referred to collectively as dioxins and dioxin-like compounds, induce a common suite of effects and have a common mechanism of action mediated by binding of the polychlorinated ligand to a specific high-affinity cellular protein. This group of dioxins and dioxin-like chemicals includes seven PCDD congeners, 10 PCDF congeners, and 12 PCB congeners. The prototype for the dioxins is 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin or TCDD. Toxicity and persistence of the polychlorinated compounds are determined by structure, with lateral substitutions on the ring resulting in the highest degree of toxicity. For the PCDDs and PCDFs, congeners with chlorines in the 2, 3, 7 and 8 positions fall into this category. The dioxin-like PCB congeners are the non-ortho- and mono-ortho-substituted compounds with no chlorines or one chlorine, respectively, on the 2, 2’, 6 or 6’ position (Safe, 1990; Headrick, 1999; Huwe, 2002; Mandal, 2005; Schecter et al., 2006).

Mechanistic studies indicate that the toxic and biochemical effects associated with exposure to TCDD and its approximate stereoisomers are mediated by initial binding of the chemical to the cytosolic aryl hydrocarbon receptor (AhR) present in target tissues and organs. There is a correlation between the AhR binding affinity of these chemicals and their structure-toxicity relationships, which supports the idea that the Ah receptor is involved in the mediation of responses induced by the TCCD-like PCDD, PCDF and PCB congeners (Okey et al., 1994; Hahn, 1998, 2002; Safe, 1998; Denison et al., 2002; Denison and Nagy, 2003; Mandal, 2005).
The common mechanism of action of TCDD and related compounds has led to the use of a toxic equivalency factor (TEF) approach to estimate the TCDD-like toxicity of complex mixtures containing chemicals that resemble TCDD. The TEF is a consensus value based on multiple data sets in an attempt to predict the potency of an individual congener relative to TCDD. Using the TEF concept, TCDD toxic equivalents (TEQs), which is the sum of the product of the concentration of each congener and its respective TEF, can be calculated for any complex mixture containing TCDD-like chemicals to provide an estimation of the total TCDD-like toxicity (Safe, 1998; Huwe, 2002).

Commercial production of PCBs, primarily by the Monsanto Corporation, began in the United States in 1929 until 1977, (Tanabe, 1988; Kimbrough, 1987, 1995; Headrick et al., 1999). They were used in closed use systems including electrical transformers, capacitors and heat transfer and hydraulic systems. For a period of time, PCBs also had a large number of open-ended applications. They were used in paints, polymers, and adhesives, as lubricants, plasticisers, fire retardants, immersion oils, vehicles for pesticide application and as agents for the suspension of pigments in carbonless copy paper (Safe, 1990; Headrick et al., 1999). The PCB products that were manufactured by Monsanto in the United States had the trade name Aroclor followed by four digits that identified the particular mixture. The first two digits referred to the 12 carbon atoms of the biphenyl molecule and the last two digits referred to the percentage of chlorine in the mixture, by weight. Aroclors 1221, 1232, 1242, 1254 and 1260 were the commercial PCB products that were produced by Monsanto, containing 21, 32, 42, 54 and 60% chlorine by weight, respectively. Similar commercial PCB mixtures were produced by other manufacturers worldwide including the Clophens (Bayer, Germany), Pheoclor and Pyralenes (Prodelec, France), Fenclors (Caffro, Italy) and Kanechlor (Kanegafuchi, Japan) (Kimbrough, 1987, 1995; Safe, 1994).

The physical and chemical properties of PCBs, such as high stability, inertness and dielectric properties, that were advantageous for many industrial purposes, led to the international use of PCBs in large quantities (Tanabe, 1988). For example, the estimated cumulative production of PCBs in the United States between 1930 and 1975 was 700,000 tonnes, and worldwide production was approximately 1.2 million tons. Domestic sales of PCBs in the United States during this time period totalled 627,000 tons (Kimbrough, 1987, 1995; Tanabe, 1988). As a result of widespread use, PCBs were identified in environmental media and biota as early as the 1960s. After the discovery of their widespread environmental contamination in the 1970s, PCB production decreased and eventually ceased (Tanabe, 1988). In 1971, Monsanto voluntarily stopped production of PCBs for open-ended uses and subsequently only the lower chlorinated biphenyls were produced (Aroclor 1242 and 1016). In 1977, Monsanto ceased production entirely (Kimbrough, 1987, 1995). Although PCBs are no longer used commercially because of their persistence, they are still present in the environment. About 31% of all the PCBs produced are present in the global environment. It is estimated that 780,000 tons are still in use in older electric equipment and other products, deposited in landfills and dumps or in storage (Tanabe, 1988).

Polychlorinated biphenyls were first detected in the Great Lakes in 1968. Since the cessation of PCB production in 1977, PCB concentrations in sediments of all the Great Lakes have either levelled off or declined. Li et al. (2009) estimate a current total accumulation of approximately 300 metric tons of PCBs in the sediment of the Great Lakes, which is 30% less than the 1980 estimate. However, Li and associates (2009) state that even if PCB degradation were substantial and widespread, the ultimate elimination of PCBs from the Great Lakes would take decades or centuries to complete. Furthermore, He et al. (2001) concluded from a 20-year survey of trends in sport fish consumption that serum PCB concentrations among consumers of sport-caught Great Lakes fish increased in the 1970s and did not subsequently decline in the 1980s, presumably because of continued low-level exposure through fish consumption and the long half-life of PCBs. Hickey et al. (2006) reported on trends of chlorinated organic contaminants, including PCBs, in Great Lakes trout and walleye from 1970 to 1998. They concluded that although concentrations of most contaminants in predator fish in the Great Lakes have continued to decrease during the 1990s, the rapid decrease in concentrations observed through the 1970s and 1980s has slowed. Furthermore, concentrations of several contaminants, including PCB
congener 126 (3,3’, 4,4’, 5-pentachlorobiphenyl), which is the most toxic dioxin-like PCB congener, appear to have stabilised. Kannan et al. (2000) reported the results of a comprehensive analysis of PCB congeners for fish originating from Michigan waters, including the Great Lakes. Concentrations of total PCBs in walleye and carp were approximately double the US Food and Drug Administration recommended tolerance limit of 2,000 ng/g, weight wet (ww) for human consumption. When expressed on a TEQ basis (Van den Berg et al., 1998), which reflects the dioxin-like toxicity, fish fillets contained from 0.46 to 58 pg TEQ/g (ww) derived from PCBs, with PCB 126 accounting for 50 to 83% of the TEQs.

Polychlorinated dibenzo-\(p\)-dioxins and PCDFs have never been produced for commercial sale. Their presence in the environment is the result of their formation as by-products of commercial and natural processes (Safe, 1990). Some of the important industrial sources of PCDDs and PCDFs have included their formation as by-products in the production of PCBs, chlorinated phenols and chlorinated phenol-derived chemicals, hexachlorobenzene, technical hexachlorocyclohexanes, and chlorides of iron, aluminium and copper. PCDDs and PCDFs have also been identified in effluents, wastes and pulp samples from the pulp and paper industry and in finished paper products. Emissions from municipal and hazardous waste incinerators as well as home heating systems that use wood and coal, diesel engines, forest and grass fires and agricultural and backyard burning contain PCDDs and PCDFs. Another contribution might come from naturally formed PCDDs and PCDFs, which have been detected in deep soils and clays from the southern United States and Germany (Safe, 1990; Huwe, 2002).

Historical data suggest that PCDDs and PCDFs entered the environment in the 1930s and 1940s, with releases peaking in the 1970s. Since the 1970s, emissions and abundance have been decreasing (Bhavsar et al., 2008). The United States Environmental Protection Agency (EPA) estimated that annual emissions of PCDDs and PCDFs decreased by 75% from 13.5 to 2.8 kg TEQ/year between 1987 and 1995. This was due primarily to improvements in incinerator performance and removal of incinerators that could not meet emission standards. Other regulations, including bans or restrictions on the production and use of chemicals such as the wood preservative pentachlorophenol (PCP), the phase-out of leaded gasoline that contained halogenated additives, and the elimination of chlorine bleaching in the pulp industry also contributed to reducing concentrations of PCDDs and PCDFs (Huwe, 2002). There has been a further 50% decline in emissions between 1995 and 2000 from known sources in the US (Bhavsar et al., 2008). Bhavsar and associates (2008) measured the concentrations of 17 of the most toxic PCDDs and PCDFs in lake trout or lake whitefish collected between 1989 and 2003 from the Canadian Great Lakes. The results of the study indicate that PCDDs and PCDFs are ubiquitous in lake trout and white fish in the Canadian Great Lakes, with the greatest concentrations occurring in Lake Ontario fish (54 pg TEQ/g in 1989 and 22 pg TEQ/g in 1999). TEQ concentrations in lake trout from Lakes Superior and Huron are 60 to 95% less than those from Lake Ontario. Temporal trend data suggest that TEQ concentrations are decreasing in Lake Ontario lake trout at approximately 1.5 pg/g/yr. TEQ concentrations also appear to be decreasing in Lake Huron lake trout, while they have stabilised in Lake Superior lake trout.

Despite a substantial drop in the emissions and environmental concentrations of PCDDs and PCDFs as well as the decline in environmental concentrations of total PCB concentrations, these chemicals are currently responsible for a substantial proportion of sport fish consumption limit recommendations for both the Canadian and US Great Lakes (Bhavsar et al., 2008). In addition, the TEQ concentrations associated with PCBs, PCDDs and PCDFs in the Great Lakes basin continue to be within a range associated with potential deleterious effects in a number of wildlife species.

**Sentinel Species**

Because humans and vertebrate species that share the same environment often have similar responses resulting from exposure to toxic substances, certain animal species can be used to monitor environmental contaminant exposure and effects. These species are considered to be sentinel species, which are defined by Grove et al. (2009) as those that are used to evaluate environmental
contamination and its implications on environmental health based on their chemical sensitivity, position in the biotic community, exposure potential and geographical distribution or abundance. For an animal to serve as a sentinel species it must meet certain requirements as presented in Basu et al. (2007). These criteria include (1) widespread distribution, (2) high trophic status, (3) ability to accumulate contaminants, (4) maintained and studied in captivity, (5) captured in sufficient numbers, (6) restricted home range, (7) well-known biology, and (8) sensitive to contaminants. The mink (*Mustela vison*) is a wildlife species that satisfies all of the above criteria. It is a fish-eating mammal that, because of its high trophic status, accumulates numerous contaminants of concern including PCBs, PCDDs and PCDFs, as indicated by numerous field (Haffner et al., 1998; Millsap et al., 2004; Martin et al., 2006a) and laboratory studies (Ringer et al., 1972; Hochstein et al., 1988, 1998, 2001; Tillitt et al., 1996; Halbrook et al., 1999; Bursian et al., 2006a,b,c; Martin et al., 2006b). Agencies and organisations such as the US Environmental Protection Agency, US Fish and Wildlife Service, US National Academy of Sciences, Environment Canada and the Swedish Environmental Protection Agency recognise the mink as a sentinel species (Basu et al., 2007), making it one of the most commonly selected receptors in ecological risk assessments for sites involving aquatic habitats with elevated concentrations of PCBs, PCDDs, PCDFs and related compounds (Blankenship et al., 2008).

**Effects of PCBs, PCDDs and PCDFs on Mink**

The identification of the mink as a potential sentinel species and its relation to the Great Lakes can be traced back to 1968, when coho salmon collected from Lake Michigan tributaries during the 1967 spawning run and incorporated into mink feed, which was fed prior to and during the breeding and whelping periods on commercial fur farms, resulted in abnormally high kit mortality (Aulerich et al., 1971, 1973). Studies conducted at Michigan State University by Richard Aulerich and associates confirmed that Lake Michigan coho salmon, when fed to breeder mink at 30% of the diet, caused either complete reproductive failure or increased kit mortality. Chlorinated pesticide (DDT, DDT isomers and dieldrin) contamination was ruled out by mink feeding experiments that indicated no reproductive effects at dietary concentrations in excess of what was detected in the fish (Aulerich et al., 1971, 1973). Further analysis of the fish indicated the presence of PCBs at concentrations up to 15 parts per million (ppm) or µg/g tissue.

Subsequent feeding studies by Aulerich and colleagues demonstrated that mink were very sensitive to PCBs. Thirty µg/g of commercial PCB mixtures (10 µg each of Aroclors 1242, 1248 and 1254) in feed proved lethal to adult breeders within four months. Fifteen µg/g feed caused reproductive failure and some adult mortality. Diets containing 10 µg of a commercial PCB mixture (Aroclor 1254)/g feed resulted in reduced weight gain when fed continuously to growing mink. The clinical signs and gross lesions, which included anorexia, bloody stools, fatty livers and haemorrhagic gastric ulcers, of mink fed diets containing the commercial PCB mixture were very similar to the signs noted in mink fed feed containing coho salmon (Aulerich et al., 1973; Aulerich and Ringer, 1977).

Subsequent studies with commercial Aroclor mixtures showed that a dietary concentration of 2 µg Aroclor 1254/g feed adversely affected reproduction based on the number of females whelping and litter size, but similar dietary concentrations of Aroclors 1221, 1242 and 1016 had no effect on reproduction (Aulerich and Ringer,
1977). Based on the results of this study, the no observed adverse effect level (NOAEL) and lowest observable effect level (LOAEL) for Aroclor 1254 based on reproductive impairment is 1 and 2 µg/g feed, respectively. A dietary NOAEL and LOAEL of 10 and 25 µg/g feed has been reported for Aroclor 1016 based on decreased kit weight at 4 weeks of age (Aulerich and Ringer, 1980). Bleavins et al. (1980) reported that a dietary concentration of 5 µg Aroclor 1242/g feed resulted in complete reproductive failure in that no bred females in this group whelped. Because this was the least dietary concentration used, a NOAEL cannot be determined.

A number of mink feeding studies have been conducted utilising fish collected from waters associated with the Great Lakes basin. Hornshaw et al. (1983) reported a reduction in litter size and kit survival through four weeks of age in mink fed diets containing yellow perch from northern Lake Erie and white suckers from Saginaw Bay, Lake Huron. The LOAEL for this study was 0.66 µg total PCBs (tPCBs)/g feed. Heaton et al. (1995) fed ranch mink diets containing up to 40% carp collected from Saginaw Bay, Lake Huron. These diets provided tPCB concentrations ranging from 0.72 µg/g feed (10% carp) to 2.56 µg/g feed (40% carp). The LOAEL in this study was 0.72 µg tPCBs/g feed (10% carp) based on reduced kit survival and body weight. Bursian et al. (2006a) conducted a study similar to that by Heaton et al. (1995) in that mink were fed diets containing carp collected from the Saginaw River, which empties into Saginaw Bay. They reported that inclusion of up to 30% carp in the feed, which provided 1.7 µg tPCBs/g feed, had no significant effect on reproduction and offspring survivability and growth. Diets in the studies by Heaton et al. (1995) and Bursian et al. (2006a) were analysed for individual PCB, PCDD and PCDF congeners, which allowed for calculation of TCDD toxic equivalents. In the Heaton et al. (1995) study, the LOAEL of 0.66 µg tPCBs/g diet corresponds to 16.8 pg TEQ/g diet using 2005 World Health Organization (WHO) TEF values (Van den Berg et al., 2006) and the reproductive NOAEL of 1.7 µg tPCBs/g diet in the Bursian et al. (2006a) study is equivalent to 56.6 pg TEQ/g diet.

One complication of feeding mink diets containing fish collected from contaminated waters is that it is probable that other unaccounted for contaminants are influencing the overall toxicity of the mixture. Furthermore, it is possible that the PPCB/PCDD/PCDF congener profile is influencing toxicity in a way that is not accounted for using the TEF approach. This may explain why Heaton et al. (1995) reported effects on kit survival and growth at dietary tPCB and TEQ concentrations that were 2.5-fold and 3.4-fold less, respectively, than the NOAEL reported by Bursian et al. (2006a). Similarly, Bursian et al. (2006 b) reported that diets containing 3.7 µg tPCB/g feed or 50.4 pg TEQ/g feed provided by carp collected from the Housatonic River, Massachusetts, caused reduced kit survivability, resulting in a LOAEL that is 5.6 and 3 times greater than the LOAEL reported by Heaton et al. (1995) when expressed on a tPCB and TEQ basis, respectively.

There have been a few mink studies conducted with single PCB, PCDD and PCDF congeners that avoid the complexities associated with exposure to a mixture of congeners as well as other contaminants. Hochstein and associates conducted studies that involved exposure of mink to TCDD. The first study (Hochstein et al., 1988) established a 28-day LD$_{50}$ of 4.2 µg TCDD/kg body weight. A 28-day LC$_{50}$ of 4.8 ng TCDD/g feed and a 125-day LC$_{50}$ of 0.85 ng TCDD/g feed was reported in the second study (Hochstein et al., 1998). Beckett et al. (2008) reported that dietary concentrations of 24 and 2.4 ng 3,3',4,4',5-pentachlorobiphenyl (PCB 126)/g feed resulted in complete reproductive failure (2,400 and 240 pg TEQ/g feed, respectively), while a dietary concentration of 0.24 ng PCB 126/g feed (24 pg TEQ/g feed) was the NOAEL. Interestingly, Zwiernik et al. (2009) reported that an equivalent TEQ concentration (242 pg/g feed) provided by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) had no effect on reproduction and survivability of offspring. Blankenship and associates (2008) conducted a comprehensive review of mink feeding studies involving PCBs, PCDDs and PCDFs, including those studies mentioned above, in order to derive dietary and tissue residue-based toxicity reference values (TRVs) expressed as TEQs. Developmental and reproductive effects were considered to be ecologically relevant endpoints. Dietary TRVs ranged from 12 to 57 pg TEQ/g feed for the NOAEL and from 50 to 242 pg TEQ/g feed for the LOAEL. The authors stated that the effects of PCDFs could not be accurately predicted from the use of TEQ-based TRVs developed from studies using PCBs or PCDDs.
Mandibular and Maxillary Squamous Epithelial Proliferation

While effects on mink reproduction and offspring survivability and growth are generally considered to be the important ecologically relevant endpoints (Blankenship et al., 2008), mandibular and maxillary squamous epithelial proliferation in the mink has attracted the attention of regulators as a potential endpoint indicative of compromised health. Render et al. (2000a) observed maxillary and mandibular osteoinvasive squamous epithelial proliferation in 12-week-old mink fed a diet containing 24 ng PCB 126/g feed. Gross examination revealed mandibular and maxillary lesions consisting of swollen tissue of the lower and upper jaws with nodular proliferation of the gingiva and loose teeth with increased gingival surface area. Radiographs indicated osteolysis of the maxilla and mandible, and histological examination documented extensive osteoinvasion by squamous epithelial cells (see Figures 16.3 to 16.5). A subsequent study by Render et al. (2001), in which 6- and 12-week-old mink were fed 24 ng PCB 126 or 2.4 ng TCDD/g feed, verified induction of maxillary and mandibular osteoinvasive squamous epithelial cell proliferation by PCB 126 and demonstrated its induction by TCDD. This latter study also showed that the lesion could be detected histologically after only two weeks of dietary treatment. Examination of adult female mink that were fed 5.0 ng TCDD/g feed for six months indicated no gross abnormalities of the maxilla or mandible, but histologically there was proliferation of squamous epithelial cells (Render et al., 2000b). The proliferation resulted in focal loss of alveolar bone or osteolysis, but not to the extent that was observed in 6- and 12-week-old mink fed PCB 126 or TCDD. The studies by Render et al. (2000a, b, 2001) suggested that juvenile mink exposed to PCB 126 or TCDD are more susceptible to proliferation.
of squamous epithelia than adult mink. Mink exposed to PCBs/PCDDs/PCDFs through consumption of diets containing contaminated fish also had histological evidence of the lesion.

Bursian et al. (2006c) reported that juvenile mink that were exposed from conception through 31 weeks of age to tPCBs concentrations as low as 0.96 µg/g feed (9.2 pg TEQ/g) provided by fish collected from the Housatonic River developed mandibular and maxillary squamous cell proliferation. Maxillary and mandibular squamous epithelial proliferation was evident in four of seven juveniles exposed from conception through to 27 weeks of age to 1.1 µg tPCBs/g feed (36.5 pg TEQs/g) and in six of eight juveniles exposed to 1.7 µg tPCBs/g feed (56.6 pg TEQs/g) derived from carp collected from the Saginaw River (Bursian et al., 2006a).

Beckett et al. (2005) reported on the incidence of mandibular and maxillary squamous epithelial proliferation in mink trapped in the Kalamazoo River area of concern (KRAOC). The Kalamazoo River Superfund site includes approximately 125 km of the Kalamazoo River from the city of Kalamazoo in southwestern Michigan, USA to Lake Michigan. This area became contaminated with PCBs by waste discharge from the recycling and processing of carbonless copy paper. Four of nine mink collected from the KROAC had histological evidence of the lesion, while mink trapped in an upstream reference area did not have the lesion. Lesion severity was positively correlated with hepatic tPCB and TEQ concentrations in mink collected from the KROAC. Hepatic tPCB and TEQ concentrations in mink having the lesion ranged from 2.9 to 6.0 µg/g and 0.21 to 1.3 pg/g, respectively.

### Contaminant Load in LGL Mink

Because of recognition of the mink as a sentinel species, there have been efforts to assess spatial and temporal trends of PCBs in mink residing in the Great Lakes basin. Mink trapped in areas adjacent to Lake Erie during the 1970s and 1980s were reported to have PCB concentrations in excess of those causing growth and reproductive effects in controlled exposure studies (Proulx et al., 1987; Haffner et al., 1998). Giesy et al. (1994) assessed TEQ concentrations in fish collected above and below hydroelectric dams on selected rivers that flow into Lake Michigan and Lake Huron and concluded that, on average, if more than 10% of a mink’s diet consisted of fish from these rivers, there was an increased risk of deleterious effects. Martin et al. (2006a) compared contaminant concentrations in liver tissue of mink trapped from 1998 to 2003 in the Lake Erie and Lake St. Clair basins to those of mink similarly obtained in 1978/1979. They reported...
that while concentrations of PCBs and other chlorinated hydrocarbons in mink generally decreased over the past two decades, PCB concentrations tended to increase in western Lake Erie mink over the same time period. Furthermore, hepatic PCB concentrations in mink were within the range associated with reproductive impairment, as determined from captive mink studies, in approximately 12% of all animals collected from the Lake Erie and St. Clair basins overall, and in almost 40% of individuals from western Lake Erie.

**Emerging Contaminants of Concern**

In addition to PCBs, PCDDs and PCDFs, there are emerging contaminants of concern that have been detected in the LGL environment. One such group of chemicals is the brominated flame retardants (BFRs), which are chemical compounds that inhibit the combustion of organic materials by scavenging free radicals that are involved in the combustion process (D’Silva et al., 2004; Hites, 2006). BFRs are incorporated in a wide variety of materials including paints, plastics, textiles, furniture and electronics by covalent bonding to the polymer or by addition into the final product (Ward et al., 2008). One type of BFR is the polybrominated diphenyl ethers (PBDEs), which gained prominence after the manufacture of polybrominated biphenyls (PBBs) was stopped in the US and Canada in the late 1970s (Hites, 2006). PBDEs are a class of additive BFRs made up of 209 possible congeners containing between 1 and 10 bromine atoms (Alaee et al., 2003). Of these 209 congeners, 23 are of environmental significance (Ward et al., 2008). Until early 2005, three commercial PBDE mixtures were widely distributed for use in North America: Pentabromodiphenyl ether (pentaBDE), octabromodiphenyl ether (octaPBDE), and decabromodiphenyl ether (decaBDE). Due to public concerns and for economic reasons, the only North American producer of octaBDE and pentaBDE voluntarily stopped producing these products in December 2004. Despite the fact that the use of penta- and octaBDE products has been effectively eliminated in North America, these chemicals will continue to enter the environment through the disposal of PBDE-containing electronics and furniture (Hites, 2006; Ward et al., 2008). In contrast, there are no restrictions on the decaPBDE commercial mixtures, which largely dominate the demand and use of PBDEs as an additive flame retardant in the global market (Gauthier et al., 2008).

More than 70,000 metric tons of PBDEs have been produced annually worldwide, 50% of which have been used in the US and Canada, including almost all of the pentaBDE manufactured (Renner, 2000; Hites, 2006). The major source of PBDEs in the LGL is atmospheric deposition (Li et al., 2006; Ward et al., 2008). PBDEs also enter the LGL through recycling and disposal of products containing PBDEs (Watanabe and Sakai, 2003; Ward et al., 2008), manufacturing output and fluvial deposition from tributaries (Samara et al., 2006; Ward et al., 2008) and wastewater treatment plants (La Guardia et al., 2007; Ward et al., 2008). Long-term studies of birds and fish in the LGL have indicated that PBDE burdens in wildlife have increased exponentially (Norstrom et al., 2002), doubling approximately every three years (Zhu and Hites, 2004). Recent trends indicate that burdens may now be levelling off in some of the lakes (Luross et al., 2000; Zhu and Hites, 2004), but if the temporal trajectory of PBDE accumulation in the LGL is maintained, concentrations of PBDEs could soon exceed the upper limit of safe levels of consumption of contaminated fish (Ward et al., 2008).

Because the mink is a fish-eating mammal that accumulates PCBs, PCDDs and PCDFs, it was not unexpected that structurally similar PBDE congeners were detected in livers of animals fed diets containing fish collected from the LGL basin. Bursian et al. (2006a) reported that 6-week-old mink kits whelped by females fed diets containing 30% carp collected from the Saginaw River had an average hepatic total PBDE concentration of 23 µg/kg. The predominant congener was BDE-47 (89%) followed by BDE-100 (5%) and BDE-209 (4%). Both BDE-47 and BDE-100 are components of DE-71, which is a commercial penta-BDE mixture, while BDE-209 is the principal component of the deca-BDE commercial mixture DE-83R.

In response to the demonstration of bioaccumulation of PBDE congeners in mink, a series of studies were conducted to determine the effects of dietary DE-71 in this sentinel species. In the initial study, juvenile mink were fed diets containing up to 10 µg DE-71/g feed for
eight weeks, which resulted in reduced feed intake and decreased body mass at dietary concentrations of 5 and 10 µg/g feed. In addition, there was an increase in antibody production, an increase in relative masses of the spleen, adrenal glands and liver and induction of liver microsomal ethoxyresorufin-O-deethylase (EROD) activity. Spleens of mink exposed to 10 µg DE-71/g feed had significantly increased germinal centre development and incidence of B-cell hyperplasia. Haematocrits in mink fed 5 and 10 µg DE-71/g feed were significantly less than in controls, while the percentage of neutrophils increased and the percentage of lymphocytes decreased (Martin et al., 2007).

To assess the effects of DE-71 on mink reproduction and development at environmentally relevant concentrations, adult female mink were fed diets containing 0, 0.1, 0.5 or 2.5 µg DE-71/g feed from 6 weeks prior to breeding until weaning of kits at 6 weeks post-parturition. Offspring in each group were maintained on their respective diets for an additional 27 weeks. A dietary concentration of 2.5 µg DE-71/g feed resulted in complete reproductive failure. Developmental effects in offspring were evident in 33-week-old juveniles, which were more sensitive to effects than their respective dams. At 0.5 µg DE-71/g feed, total triiodothyronine (T3) was significantly decreased in both male and female juveniles while thyroid follicular epithelium cell height was elevated when compared with controls. The NOAEL and LOAEL for T3 disruption based on juvenile hepatic PBDE concentrations were 1.2 and 6.4 µg/g. Hepatic EROD activity was significantly induced in all exposed offspring at 33 weeks (Zhang et al., 2009). This study also demonstrated that environmentally relevant exposures to DE-71 did not affect key parameters of the cholinergic neurotransmitter system in the brain of ranch mink (Bull et al., 2007).

These studies indicate that exposure to a commercial pentaBDE mixture can cause deleterious effects in mink. Biomonitoring of wild mink in the LGL region indicated that most populations have hepatic concentrations of total PBDEs that are currently less than those expected to affect thyroid hormone homeostasis, but margins of safety are small and hepatic PBDE concentrations in mink around Hamilton Harbour, Ontario exceeded the NOAEL for T3 disruption (Zhang et al., 2009).

Conclusions

Chemical contaminants have had deleterious effects on numerous wildlife species residing in the LGL basin over the past half-century. In many cases, awareness of the negative impact has resulted in a reduction or discontinuation of the use of many of these chemicals. This in turn has led to a partial, if not full, recovery of certain species. We should point out, however, that some of these historical contaminants, such as PCBs, continue to occur in concentrations that can induce effects in sensitive species such as the mink, despite the fact that production of PCBs ceased over 30 years ago. Furthermore, contaminants that have been detected relatively recently, such as the brominated flame retardants, continue to increase in the LGL, approaching concentrations that have been demonstrated in laboratory studies with mink to cause potentially deleterious effects. Considerably more research is needed to better characterise the occurrence of these chemicals in the LGL and their effects on wildlife so that effective measures can be taken to reduce the impact of the emerging contaminants of concern on this vital resource.
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