LAKE ERIE LaMP BENEFICIAL USE IMPAIRMENT ASSESSMENT: ANIMAL DEFORMITIES AND REPRODUCTION IMPAIRMENT

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EXECUTIVE SUMMARY

Under the Great Lakes Water Quality Agreement, Lakewide Management Plans (LaMPs) have been charged with evaluating impairments of beneficial uses of various natural resources caused by priority pollutants. This report assessed deformities and reproductive impairments in wildlife species of Lake Erie. Population-level impairments were covered in a separate assessment. Impairments were assessed using epidemiological criteria, species-specific biological criteria for deformity rates and reproductive success and species-specific chemical criteria for tissue concentrations associated with reproductive effects in field and (or) laboratory studies.

Bald Eagles nesting within eight km of Lake Erie have impaired reproduction. The Ohio Lake Erie eagles remain below the recovery goal of 1.0 young fledged/occupied nest. Increases in the reproductive success of the Lake Erie basin Bald Eagle subpopulation may have been influenced by several factors that confound evaluation of contaminant effects. Hacking and fostering projects along the Ontario and Ohio shorelines during the mid-1980s introduced individuals that were not exposed to Lake Erie contaminants as embryos. The recovery of the Bald Eagle throughout North America has created a pool of young eagles dispersing to find unclaimed breeding territories. The high turnover of adults along the Ohio and Michigan shorelines of Lake Erie suggests the possibility of colonization of these territories by eagles raised elsewhere. Eagle eggs from the Ohio and Ontario shorelines of Lake Erie consistently exceed PCB and dieldrin criteria and often exceed the p,p'-DDE criterion for adverse reproductive effects. The rate of deformities in Lake Erie eagles is greatly above the background rate for birds and is similar to the rate in cormorants in Green Bay, which is among the highest reported rates for birds.

PCB concentrations in eggs of fish-eating colonial waterbirds such as Herring Gulls, Common Terns, and Double-crested Cormorants consistently exceed levels associated with embryonic mortality and (or) deformities, especially in the western basin. DDE-induced eggshell thinning does not appear to be important at current levels of contamination for any of the colonial waterbirds examined.

Some assessments of numbers of trapped mink suggest lower mink populations along Lake Erie compared to inland areas, but other assessments show little difference from inland areas. Data on Lake Erie mink tissue residues and food items indicate that wild mink are exposed to potentially harmful concentrations of PCBs. Otters were extirpated from the Lake Erie watershed by 1900 and there are no data to support or refute an association between PCBs and reproduction and population impairments in Lake Erie, although data from other locations indicate a cause-effect relationship. Exposure to PCBs is often greater for otters than mink and otters may be equally sensitive to the effects of PCBs.

Organochlorine concentrations in Lake Erie water snakes, eastern spiny softshell turtle eggs, and some snapping turtle eggs strongly suggest reproductive effects. The presence of p,p'-DDE in some amphibians from Point Pelee suggests that further study is required to determine the sensitivity to p,p'-DDE of amphibian species native to Lake Erie. Nitrate concentrations in agricultural watersheds of Lake occasionally exceed concentrations associated with mortality and

reduced growth and development in laboratory studies of amphibian tadpoles. The use of TFM, which is intended to control larval lamprey, is likely to kill amphibians wherever it is used in Lake Erie tributaries. Deformity rates in mudpuppies at Long Point and in the Detroit River are elevated well above the background rates reported for inland areas of the Great Lakes and St. Lawrence River basin.

A risk assessment using species-specific biomagnification factors was conducted to compare ambient water concentrations of PCBs to concentrations that have been shown to have no effect in laboratory and (or) field studies. For all species assessed (Bald Eagles, Herring Gulls, Double-crested Cormorants, Caspian Terns and mink), PCB concentrations in Lake Erie water were nine to 1,550 fold higher than no effect concentrations.

SOMMAIRE EXÉCUTIF

En vertu de l'Accord relatif à la qualité de l'eau dans les Grands Lacs, les responsables des plans d'aménagement pan lacustre sont chargés d'évaluer les atteintes aux utilisations des ressources naturelles attribuables aux polluants d'intérêt prioritaire. Le présent rapport examine les difformités et les problèmes de reproduction chez les espèces fauniques du lac Érié. Les effets sur le niveau des populations ont fait l'objet d'une analyse distincte. Les atteintes ont été évaluées en fonction de critères épidémiologiques, de critères biologiques spécifiques d'espèce pour les taux de difformités et le succès de la reproduction, et de critères chimiques spécifiques d'espèce pour les concentrations tissulaires associées aux effets sur la reproduction dans des études sur le terrain et(ou) en laboratoire.

Les pygargues à tête blanche qui nichent dans un rayon de huit km du lac Érié affichent des problèmes de reproduction. En Ohio, les populations de pygargues du lac Érié restent en deçà de l'objectif de rétablissement de 1,0 oisillon apte au vol/nid occupé. L'accroissement du succès de la reproduction chez la sous-population de pygargues à tête blanche du bassin du lac Érié pourrait être dû à des facteurs multiples, ce qui complique l'évaluation des effets des contaminants. Les projets de lâcher et d'adoption intra spécifique de pygargues le long des rives du lac en Ontario et en Ohio au milieu des années 1980 ont permis d'introduire des individus qui, à l'état d'embryons, n'avaient pas été exposés aux contaminants du lac Érié. Le rétablissement du pygargue à tête blanche sur tout le continent nord-américain a favorisé la dispersion de jeunes pygargues à la recherche de territoires de nidification inoccupés. La hausse marquée de l'effectif adulte le long des rives du lac Érié en Ohio et au Michigan laisse présumer une colonisation possible de ces territoires par des pygargues provenant d'autres régions. Les concentrations de PCB et de dieldrine mesurées dans les oeufs de pygargues prélevés sur les rives du lac Érié en Ohio et en Ontario sont régulièrement supérieures à celles précisées dans les critères et dépassent souvent les concentrations de p,p'-DDE ayant des effets négatifs sur la reproduction. Le taux de difformités chez les pygargues du lac Érié est nettement supérieur au taux habituel observé chez les oiseaux et correspond plus ou moins à celui observé chez les cormorans de la baie Green, qui est parmi les plus élevés chez les oiseaux.

Les concentrations de PCB dans les oeufs des oiseaux de rivage coloniaux comme le goéland argenté, la sterne pierregarin et le cormoran à aigrettes dépassent constamment les concentrations associées à une mortalité embryonnaire et(ou) des malformations, notamment dans l'ouest du bassin. L'amincissement de la coquille des oeufs provoqué par le DDE ne semble pas être important aux niveaux actuels de contamination chez aucun des oiseaux de rivage coloniaux examinés.

Des évaluations du nombre de visons capturés laissent présumer que les populations de l'espèce sont plus faibles le long du lac Érié que dans les secteurs de l'intérieur, mais d'autres évaluations montrent peu de différences par rapport aux secteurs de l'intérieur. L'analyse des résidus dans les tissus et des aliments consommés par les visons du lac Érié révèle que les visons sauvages sont exposés à des concentrations potentiellement nocives de PCB. Les loutres avaient disparu du bassin versant du lac Érié au tournant du XX^{e} siècle, et nous ne disposons d'aucune donnée pour confirmer ou infirmer l'existence d'une corrélation entre les PCB et les problèmes de reproduction et le déclin des populations dans le lac Érié, bien que des données recueillies à d'autres endroits révèlent des liens de cause à effet. Les loutres sont souvent plus exposées aux PCB que les visons et pourraient être aussi sensibles aux effets des PCB.

Les concentrations d'organochlorés dans les tissus des serpents d'eau du lac Érié, les oeufs des tortues à carapace molle et les oeufs de certaines chélydres serpentines suggèrent fortement des effets sur la reproduction. La présence de p,p'-DDE chez certains amphibiens de la pointe Pelée laisse supposer qu'il faudra faire d'autres études pour déterminer la sensibilité au p,p'-DDE des espèces d'amphibiens indigènes du lac Érié. Les concentrations de nitrates dans les bassins versants agricoles du lac dépassent parfois celles associées à une mortalité ainsi qu'à un taux de croissance et de développement anormal dans les études en laboratoire réalisées sur des têtards d'amphibiens. L'emploi de TFM dans la lutte contre la lamproie marine causera probablement la mort d'amphibiens partout où ce lampricide est utilisé dans les tributaires du lac Érié. Les taux de difformités chez les nectures tachetés de Long Point et de la rivière Detroit sont nettement supérieurs aux taux normalement observés dans les secteurs de l'intérieur du bassin des Grands Lacs et du Saint-Laurent.

Une évaluation du risque basée sur les facteurs de bioamplification spécifiques d'espèce a été faite pour comparer les concentrations ambiantes de PCB dans l'eau aux concentrations sans effet mesurées en laboratoire et(ou) sur le terrain. Pour toutes les espèces évaluées (pygargue à tête blanche, goéland argenté, cormoran à aigrettes, sterne caspienne et vison), les concentrations de PCB dans l'eau du lac Érié étaient de neuf à 1 550 fois supérieures aux concentrations sans effet.

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This technical report was prepared to provide the scientific basis for the impairment conclusions recorded in the Lake Erie Lakewide Management Plan 2000 Report. The authors acknowledge the extensive review by the government agencies that are partnering to produce the LaMP, outside experts and the Lake Erie LaMP Public Forum, a group of citizen volunteers. Their review was designed to answer two questions: a) Is the document technically sound and defensible and b) do the reviewers agree with the conclusions regarding impairment. In its present form, this report has been revised to address the comments received during that review process and there is majority agreement by the reviewers with the impairment conclusions presented.

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LIST OF ABBREVIATIONS

AOC	Area of Concern
BCF	bioconcentration factor
BMF	biomagnification factor
DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
EC_{50}	effective concentration for producing an effect in 50% of the population
GLEMEDS	Great Lakes Embryo Mortality Edema and Deformities Syndrome
IJC	International Joint Commission
LaMP	Lakewide Management Plan
LC_{50}	lethal concentration for 50% of the population
$96h\ LC_{50}$	lethal concentration required to kill 50% of the test population within 96 hours
LOEC	lowest observed effect concentration
NOEC	no observed effect concentration
OC	organochlorine
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyl
TFM	3-trifluoromethyl-4-nitrophenol

1. INTRODUCTION

Industrial activity, agriculture, and development have led to many changes in the Lake Erie ecosystem. During the 1960s and 1970s, pollution was associated with reproductive failures and population declines in many species of fish-eating wildlife (Grasman *et al.* 1998). Since the 1970s, many problem pollutants have been restricted or banned, but additional chemicals have been introduced into Lake Erie. Furthermore, significant changes in the aquatic community, such as the introduction of the zebra mussel (*Dreissena polymorpha*) and other exotic species, have changed how energy and potentially pollutants move through the food web. As part of the Lake Erie Lakewide Management Plan (LaMP), it is important to:

- assess the current reproductive status of Lake Erie wildlife to determine whether impairments continue;
- assess the causes of these impairments whenever possible; and
- identify important data gaps concerning reproduction and deformities in Lake Erie wildlife.

For each species addressed in this assessment, the primary questions to be answered were: "Is the reproductive health of this species impaired and what is the extent of the impairment?" Whenever possible, current reproductive status was compared to species-specific criteria for reproductive success.

Reproductive impairments cannot be understood clearly without considering the factors that cause them. Determining the cause(s) of the impairments, whenever possible, was a secondary purpose of this assessment. However, most of the data describing impairments have been collected in toxicological studies directed at determining the causes of the problems, especially pollutants. Ideally, large monitoring studies of reproduction or population status would be used to determine impairment first. Subsequently, additional investigations would then look for the factors causing the impairment. However, in some cases much of the evidence for impairment comes from a risk assessment (e.g., comparing egg or diet residues to a reference dose), which by its definition includes a potential causal factor. If such a risk assessment suggests reproductive impairment, then that is part of the weight of evidence for impairment. It does not rule out other factors (non-chemical or other chemicals) that might contribute to the impairment. This report also addresses the level of confidence that can be ascribed to the status and potential cause(s) of the impairment. This aspect included assessment of the quality of the data and identification of data gaps and research needs.

2. SUMMARY OF REPRODUCTIVE AND DEFORMITY IMPAIRMENT CONCLUSIONS

2.1 Reproductive Impairments

2.1.1 Bald Eagle

Bald Eagles nesting within eight km of Lake Erie have impaired reproduction. Although Bald Eagle productivity and the number of fledglings (survival to age of flying) per nest has increased since 1980, the Ohio Lake Erie eagles remain below the recovery goal of 1.0 young fledged per occupied nest. Increases in the reproductive success of the Lake Erie basin Bald Eagle subpopulation may have been influenced by several factors that potentially confound linkages between exposure to contaminants in Lake Erie and biological effects. A large number of uncontaminated nestlings were introduced to the subpopulation through hacking and fostering projects along the Ontario and Ohio shorelines during the mid-1980s. There appears to have been a great increase in nesting success within the time period associated with the sexual maturation of these introduced birds. The recovery of the Bald Eagle throughout North America, including inland areas near the Great Lakes, has created a pool of young eagles dispersing to find available and unclaimed breeding territories. The high turnover of adults along the Ohio and Michigan shorelines of Lake Erie suggests the possibility of colonization of these territories by eagles raised elsewhere. Reproductive success tends to increase following replacement of an older adult in a shoreline territory.

Because human interventions and natural immigration potentially have led to the introduction of adult eagles that were not exposed developmentally (i.e., *in ovo*) to Lake Erie contaminants, a risk assessment based on comparison of contaminant concentrations in eagle eggs to known adverse effect concentrations is particularly insightful. Eagle eggs from the Ohio and Ontario shorelines of Lake Erie consistently exceed PCB and dieldrin criteria and often exceed the p,p'-DDE criterion for adverse effects in this species. These Lake Erie data, when interpreted in the context of studies showing effects of organochlorines on eagle reproduction elsewhere in North America, strongly suggest that current concentrations of organochlorines in Lake Erie are causing reproductive impairment of Bald Eagle reproduction.

2.1.2 Colonial Waterbirds

There are significant reproductive and physiological impairments in Herring Gulls from western Lake Erie. The cause of the reproductive impairment requires further investigation. Potential causes include contaminants, cyanobacterial toxin (microcystins), and infectious diseases. Many but not necessarily all of the physiological impacts, including immunosuppression and altered thyroid function and vitamin A status, are associated with PCB exposure. Current PCB concentrations in Lake Erie Herring Gull eggs, especially in the western basin (20 ug/g), are sufficient to cause some embryonic mortality (LOEC 5.0 ug/g).

While the tree nesting habits of Lake Erie cormorants make reproductive assessments difficult, PCB concentrations in their eggs suggest reduced hatching success and increased rates of deformities are occurring.

Recent data for Common Terns in the western basin indicates reproductive impairment caused primarily by predation. However, organochlorine concentrations in eggs are high enough to contribute to reproductive impairment.

Overall, PCB concentrations in Lake Erie, primarily in the sediments but also the water column, are high enough to present a significant risk of reproductive and physiological effects in fish-eating colonial waterbirds, especially in the western basin.

DDE-induced eggshell thinning does not appear to be important at current levels of contamination in any of the species examined.

2.1.3 Tree Swallows

There does not appear to be reproductive impairment in Lake Erie Tree Swallows. Tree Swallows are good indicators of exposure to contaminants via aquatic insects, but they are not particularly sensitive to the reproductive impacts, as measured in terms of embryonic survival and fledging success, of organochlorine exposure. It is possible that other insectivorous passerine species in the Lake Erie basin are more sensitive to the reproductive effects of contaminants; however, few lend themselves to monitoring as well as do Tree Swallows, and have, as yet, not been assessed.

2.1.4 Mink and River Otter

Trapping data provide our only estimates of mink population abundance. However, factors such as trapping effort and correlation with muskrat abundance confound the analysis of trapping data as an indicator of reproductive success. Some assessments of numbers of trapped mink suggest lower mink populations along Lake Erie compared to inland areas, but other assessments show little difference from inland areas.

Although strong population data do not exist, laboratory studies clearly show that mink are highly sensitive to the lethal, reproductive, and immunosuppressive effects of PCBs. The few existing data on Lake Erie mink tissue residues indicate that wild mink are exposed to potentially harmful concentrations of PCBs. Analysis of important prey species for mink suggests high concentrations of PCBs above dietary concentrations known to adversely affect mink in the laboratory, strongly indicating potential reproductive impairments.

Otters were extirpated from the Lake Erie watershed by 1900 and there are no data to support or refute an association between PCBs and reproduction and population impairments in Lake Erie, although data from other locations indicates a cause-effect relationship. Exposure to PCBs is often greater for otters than mink and otters may be equally sensitive to the effects of PCBs.

Although otters were historically present in the Lake Erie watershed and suitable habitat exists today, recovery of otter populations in the Lake Erie basin is slow, as evidenced by the relatively rare observations of otter families in the Lake Erie watershed despite reintroduction programs.

2.1.5 Reptiles

The few available studies on turtles in Lake Erie have shown that contaminant concentrations in snapping turtle eggs from Big Creek Marsh (Long Point) and Rondeau Provincial Park, Lake Erie (Canada) are not associated with elevated rates of embryo toxicity or deformities. Concentrations of PCBs detected in eggs from two sites in Ohio are similar to those from Hamilton Harbour where reproductive anomalies have been correlated with PCB exposure in snapping turtle eggs. Feminization effects noted in adult male snapping turtles from Long Point (Canada) indicate subtle endocrine disrupting effects may be occurring.

Contaminant concentrations in Lake Erie water snakes from Pelee Island are high enough to justify a study of health and reproductive effects. Contaminant concentrations in the eastern spiny softshell turtle and the corresponding low rates of hatching in eggs in the Lake Erie basin suggest further investigation of contaminant effects is warranted. This species is considered threatened provincially in Ontario and nationally in Canada.

2.1.6 Amphibians

Contaminant concentrations, including organochlorine and polycyclic aromatic hydrocarbons, have been measured in amphibians at various locations in the Canadian Lake Erie basin. Studies of biological effects are sparse. The presence of p,p'-DDE in some amphibians from Point Pelee suggests that further study is required to determine the sensitivity to p,p'-DDE of amphibian species native to Lake Erie.

Nitrate concentrations in agricultural watersheds of Lake Erie (Canada and USA) are high enough to exceed the LC_{50} (lethal concentration required to kill 50% of the test population) (3.1% of water samples) or sublethally affect (a reduced rate of growth and development; 19.8% of water samples) amphibian tadpoles of various species. However, these predictions are based on laboratory-based studies and need to be tested in wild populations.

The use of TFM (3-trifluoromethyl-4-nitrophenol), which is intended to control larval lamprey, is likely to kill amphibians wherever it is used in Lake Erie tributaries. Population effects of TFM on amphibians have been noted in the Grand River of Ohio, the only site studied to date. Because TFM is not bioaccumulative and is only applied periodically in closely controlled and monitored conditions, the associated mudpuppy mortality is often perceived to be insignificant. However, mudpuppies do not become sexually mature until four to six years of age. Given the projected schedule for future TFM applications, there is the potential for the TFM applications to match periods when large numbers of mudpuppy are reaching an age when they can reproduce. In addition, TFM is generally applied in the spring when stream flows are higher. Therefore, TFM has the potential to kill a portion of the existing females before they lay their eggs in May and June.

2.2 Deformity Impairments

The weight of evidence from field and laboratory studies shows a causal association between dioxin-like chemicals and developmental deformities such as crossed bills. In the Great Lakes, the largest proportion of dioxin-like activity is attributable to PCBs. The presence of deformities, which is an impairment under IJC listing criteria, has been shown in Lake Erie eagles and is suspected in Herring Gulls (based on anecdotal observations) and in cormorants (based on comparing PCB concentrations in Lake Erie eggs to concentrations at other Great Lakes sites where elevated deformities have been documented). The rate of deformities in Lake Erie eagles is greatly above the background rate for birds and is similar to the rate in cormorants in Green Bay, which is among the highest reported rates for birds. The observation of two deformed Herring Gulls in western Lake Erie, which has high PCB contamination, provides further evidence for impairment. The tree-nesting habits of Lake Erie cormorants precludes the examination of large numbers of chicks, but PCB concentrations in these eggs are similar to or greater than the concentrations in Green Bay cormorant eggs, so a high deformity rate is predicted for Lake Erie cormorants.

Deformity rates in mudpuppies at Long Point and in the Detroit River are elevated well above the background rates reported for inland areas of the Great Lakes and St. Lawrence River basin. However, a contaminant-associated etiology has not been confirmed at this time.

Table 1. Summary of animal deformity or reproduction impairment conclusions.

Species/ Species Group	Reproduction		Deformities		Physiology			Notes
	Impaired?	**Likely Cause**	**Impaired?**	**Likely Cause**	**Impaired?**	**Likely Cause**	**Type of Impairment**	
Bald Eagle	**Yes**; observed; exposure above effect levels	PCBs, dieldrin, DDE	**Yes**; observed	PCBs	No data			* Extent of impairment is probably obscured by hacking/fostering and immigration from less contaminated inland territories
Colonial Waterbirds	**Yes**; observed in Herring Gull; exposure above effect levels in Herring Gull, cormorant and Common Tern eggs	PCBs, possibly other chemicals	**Yes**; observed; exposure above effect levels	PCBs	**Yes**; observed; exposure above effect levels	PCBs, other organochlorines (OCs)	Immune system, reproductive organs, thyroids, liver enzymes, vitamin A and porphyrins*	* Most data from W. basin and Herring Gulls eggs ? * Tree nesting cormorants hard to study, but contaminant concentrations are among highest in Great Lakes and are likely associated with embryonic mortality and deformities ? * Cause of recent reproductive failures of Herring Gulls on W. Sister Is. may include PCBs, microcystin and (or) other factors * Although Caspian Terns have attempted to colonize Lake Erie as recently as 1996, they are still too rare in the basin for field study.
Tree Swallow	No		No data		No			* Significant OC exposure; resistance to effects may make swallow a poor indicator species for other insect-eating songbirds
Mink	Likely; PCB levels in food above effect levels	PCBs	No data		No data; likely, based on PCB levels in food			
Otter	Insufficient data, but likely based on predicted high exposure	PCBs	No data		No data			* Too rare in Erie basin for study, as they were re-introduced in 1986.
Snapping Turtle	Not observed, but exposure at some Ohio sites above effect levels	PCBs, other OCs	Not observed, but exposure at some Ohio sites above effect levels	PCBs, other OCs	Likely	organochlorines	Endocrine /reproductive	

Table 1. Summary of animal deformity or reproduction impairment conclusions (continued).

Species/ Species Group	Reproduction		Deformities		Physiology			Notes
	Impaired?	**Likely Cause**	**Impaired?**	**Likely Cause**	**Impaired?**	**Likely Cause**	**Type of Impairment**	
Frogs/Toads	Likely	High DDE and nitrates	No data		No data			* Nitrate concentrations in Lake Erie watershed often exceed lethal and sublethal concentrations for amphibians in laboratory experiments (see section 7.6.4.1.2)
Mudpuppies	Considered likely (due to TFM) by the authors but inconclusive by the LaMP committee		**Yes**; observed	PAHs and OCs, are possible causes	No data			* Data from the Grand River in Ohio and elsewhere in the Great Lakes indicate acute mortality following TFM application for lamprey control (see section 7.6.4.2)

* porphyrins - the liver synthesizes heme, which is important for hemoglobin and some enzymes. PCBs and other organochlorines block this process and cause the accumulation of intermediate products called highly carboxylated porphyrins.

3. ASSESSMENT CRITERIA AND APPROACH

According to the International Joint Commission (IJC), an animal reproductive or deformity impairment occurs when "wildlife survey data confirm the presence of deformities (e.g., cross-bill syndrome) or other reproductive problems (e.g., eggshell thinning) in sentinel wildlife species (IJC 1989)." Levels of pollutants in fish-eating wildlife and species that spend a significant part of their lives in or near the water are used as indicators of environmental conditions and these animals make good sentinel species.

Using the IJC listing criteria, the LaMP team needed to determine the types of survey data that would confirm impairments for Lake Erie. Remedial Action Plan (RAP) teams in Great Lakes Areas of Concern (AOCs) have used two endpoints to define wildlife deformity and reproductive impairments:

- when the incidence rates of cross-bill syndrome, reproductive failure, etc. are significantly (95% probability level) higher than incidence rates at control sites; or
- when Bald Eagle (*Haliaeetus leucocephalus*) reproduction is less than one eaglet per active nest.

While these impairment endpoints are appropriate for the Lake Erie LaMP, they are not comprehensive enough. Data and evaluation criteria are available for sentinel species other than the Bald Eagle. The LaMP has adopted a weight of the evidence approach to this particular assessment where information from all of the following criteria are considered, where available, to draw impairment conclusions for a particular species.

Species-specific criteria may be based on biological or chemical assessments. These species-specific criteria are based on endpoint concentrations that must not be exceeded in order to maintain unimpaired reproduction or healthy populations, or on reproductive values measured at reference sites not impacted by pollutants. Biological endpoints include measures of reproductive success necessary for a stable or growing population. Typical measures of reproductive success include rates of embryonic survival or fledging success. For example, the above-mentioned productivity standard of 1.0 young per occupied nest has been used as the recovery goal for healthy populations within the Northern States Bald Eagle Recovery Plan (Grier *et al.* 1983). Other biological criteria are discussed in the sections on each species.

Results of field and laboratory toxicity studies can be used to derive chemical criteria that must not be exceeded in order to maintain normal reproduction or a healthy population. Common examples of these endpoint concentrations for biological effects are Lowest Observable Effects Concentrations (LOECs; concentrations at which reproductive effects are statistically different from reference sites) and No-Observed Effect Concentrations (NOECs; highest observed concentrations at which reproduction is not statistically different from reference sites). See Table 13 for examples of species-specific chemical criteria and other criteria are given in the sections on each species. For example, reproductive NOEC criteria for Bald Eagles are 4.0 mg/kg for total PCBs, 3.5 mg/kg for p,p'-DDE and 0.1 mg/kg, dieldrin (all expressed as fresh, wet weight; Wiemeyer *et al.* 1984; Wiemeyer *et al.* 1993; Giesy *et al.* 1995). If chemical concentrations above these criteria are known to be present in fish and wildlife tissues in the Lake Erie ecosystem, we can conclude that impairment is likely, even if a particular population has not been monitored for reproductive effects and deformities. Such a finding is a good tool to focus future reproduction and population studies. A risk assessment based on field-derived exposure levels and species-specific effect levels avoids the uncertainties of interspecies extrapolations and estimated exposure (by modeling).

There are also important effects not mentioned in the IJC listing criteria, but which nevertheless can be used to indicate whether impairment is occurring. As the fields of biochemistry, molecular biology, and physiology have advanced during the last 25 years, biologists have gained additional tools for investigating the effects of pollutants on wildlife. Often these physiological variables are called biomarkers—biochemical, cellular, or physiological changes that indicate exposure to and toxic effects of pollutants (e.g., immune function, histology, vitamin A stores, porphyria, liver enzyme activity, reproductive hormones). Measurement of impairments within the body helps to explain deformities and reproductive problems seen on the organism level. For humans, physicians use laboratory tests to elucidate the causes and severity of particular diseases. Environmental toxicologists have successfully used biochemical and cellular biomarkers to investigate contaminant-associated impairments in fish and wildlife species, especially in the Great Lakes. Measurement of these characteristics in sentinel species can provide important supplemental information to more traditional assessments of reproductive performance and deformities.

4. SCOPE OF THE ASSESSMENT

Contaminant studies often tend to look at **effects in a particular organism in a particular location** versus population-wide effects. As per the IJC listing criteria, the current assessment is not required or intended to determine whether basin-wide or sub-population effects are occurring due to the identified deformities or reproductive problems. The purpose of our assessment was to identify whether reproduction or deformity problems are occurring due to chemical contaminants. Population-level impairments were covered in a sister Lake Erie LaMP assessment of the degradation of wildlife populations (Lambert *et al.* 2001).

Reproductive effects do not immediately or always translate into population effects. For example, if a population is near its carrying capacity (point at which species is in equilibrium with its environment), then there may not be enough resources (food, nesting habitat, etc.) for all young to survive to reproductive age. Hence, up to a point, a decrease in production of young due to a contaminant may not affect adult population size because many young would have died anyway. However, if the population is below its carrying capacity, a decrease in production of young may prevent the population from reaching carrying capacity. In this situation, the impairments summarized in Table 1 can become more significant when all stressors (contaminants, habitat loss, exotics, etc.) to a particular species group are summed.

The geographic area where impairments were identified includes the open waters of Lake Erie, the nearshore areas, embayments, river mouths and the lake effect zone of tributaries. However, the source and (or) cause of an impairment may fall outside this area. The Lake St. Clair watershed was not included in the scope of the Lake Erie LaMP.

Deformity and (or) reproductive impairments have been assessed in:

- aquatic birds, including Bald Eagles, colonial waterbirds and Tree Swallows,
- fish-eating mammals, including mink (*Mustela vison*) and river otter (*Lutra canadensis*), and
- amphibians and reptiles, including snapping turtles (*Chelydra serpentina serpentina*) and eastern spiny softshell turtles (*Apalone spiniferus*), frogs and toads in general and mudpuppies (*Necturus maculosus*).

Detailed findings for each group are presented in section 6 and Table 1.

5. EPIDEMIOLOGICAL CRITERIA FOR ASSESSING IMPAIRMENTS AND THEIR CAUSES

Epidemiologists have long recognized that cause-effect relationships can be difficult to identify, often because an effect may have multiple causes and because confounding factors may be present. In epidemiology, a factor is considered to be causal when its presence increases the probability of an effect, or, conversely, if reducing that factor decreases the occurrence of the effect. Epidemiologists often use the following criteria to evaluate possible cause-effect relationships:

(1) Consistency of Association (upon replication in different species or in different studies);
(2) Strength of Association (magnitude of the effect);
(3) Time Order (does exposure to the supposed causal factor precede the effect, or does reducing the factor lead to a subsequent decrease in the effect);
(4) Predictive Power (does the hypothesis allow the prediction of an effect in a new species or location that is subsequently verified);
(5) Statistical Significance (is the relationship between the hypothesized cause and the effect statistically significant, i.e., have a low probability of arising by chance); and
(6) Coherence (does the hypothesized cause-effect relationship make sense based on other biological and chemical information).

Not all of these criteria have to be satisfied to establish a cause-effect relationship. The epidemiologist weighs the criteria that support such a relationship against those that detract from the relationship or are indeterminate.

This approach is widely used in the field of epidemiology and has been applied successfully by Great Lakes toxicologists to evaluate potential cause-effect relationships between pollutants and impairments in wildlife (Fox 1991; 1993; Wren 1991). These criteria provide a framework for 'weighing the evidence' regarding connections between contaminants and effects.

Although originally developed for determining causation, a similar approach also can be used to determine impairments. For instance, impairment should be observed consistently (1), be of sufficient magnitude to be biologically (2) and statistically (5) significant. The impairment should be current or recent (3) and allow predictions of similar impairments in other species or locations (4). Also, if these criteria provide support for a causal relationship between some factor and impairment, there is implicit support for the existence of the impairment.

The ecoepidemiological approach was used for species for which there was sufficient information (published and unpublished sources). The peer-reviewed literature contains ecoepidemiological assessments of contaminant effects for the following Great Lakes wildlife species: Bald Eagles, colonial waterbirds, mink, river otter, and snapping turtles. Sufficient data were found to apply these criteria to eagles, colonial waterbirds, mink, and otter as part of this Lake Erie impairment assessment. For other species for which fewer data are available, a descriptive approach of impairments and potential causes was used.

6. STATUS AND POTENTIAL CAUSES OF IMPAIRMENTS

6.1 Aquatic Birds

6.1.1 Bald Eagle

Introduction

As in other areas of the Great Lakes, Lake Erie Bald Eagle populations experienced significant reproductive impairment and population declines during the 1960s and 1970s. Following bans on many organochlorine contaminants and human efforts to restore eagle populations, the size of the Lake Erie eagle population has improved (see below). This assessment focuses on impairments related to environmental toxicant exposure to Bald Eagles nesting along the shorelines of Lake Erie since 1980. This analysis is approached from four perspectives: reproductive impairment, deformities, concentrations of contaminants in eggs, and assessment of reproductive impairments using epidemiological criteria.

Reproductive Impairment

For 1980/99, Bald Eagle productivity data for all breeding areas within 8.0 km of Lake Erie were analyzed (unpublished data, P. Hunter, Ontario MNR; M. Shieldcastle, Ohio DNR; D. Best, USFWS, East Lansing). Lake Erie was divided into four regions: Ontario shoreline; Michigan shoreline; Lake Erie shoreline of Ohio; and Sandusky Bay area of Ohio. (The latter two areas are mutually exclusive.)

Productivity (i.e., total number of fledged young per occupied nest) was calculated for Bald Eagles for all breeding areas, 1980/99, for the entire lake and by region, using the method of Postupalsky (1974). Productivity within breeding areas was analyzed three ways: annually; for five-year time intervals (1980/84; 1985/89; 1990/94; 1995/99); and for 10-year time intervals (1980/89; 1990/99). Productivity within each area was determined by dividing the total number of young by the number of occupied breeding areas for each year (Postupalsky 1974). To isolate the potential effects of developmental toxins in Lake Erie on eagle reproduction, this analysis only included nestlings that had been naturally hatched within the Lake Erie region. Nestlings hacked at Long Point, Ontario, or fostered young in Ohio were excluded from these productivity rates because they were not exposed developmentally (in the egg) to Lake Erie contaminants. These productivity rates were compared to the recovery goal of 1.0 young per occupied nest from the Northern States Bald Eagle Recovery Plan (Grier *et al.* 1983).

Productivity of Bald Eagles nesting along Lake Erie has generally improved over time (Table 2). Since 1995, reproduction has, for the whole lake, been above the criteria used to indicate a healthy population, 1.0 young per occupied nest. However, there are differences among the four regions of Lake Erie, with the northern shore of Lake Erie (Ontario) having the greatest productivity (1.13 young per occupied nest) and the Lake Erie shoreline of Ohio having least (0.85 young per occupied nest).

Productivity of eagles has also generally improved when one combines the data into five-year (Table 3) and 10-year periods (Table 4). Combining the number of nesting attempts and fledged young into either five- or 10-year periods improves our ability to reduce the influence of a single event on the overall annual productivity rates due to small sample sizes. On average for each of the five-year periods, eagles nesting in Ontario have been above the healthy productivity level since 1985, the Sandusky Bay area of Ohio and the Michigan shoreline have been above the healthy productivity level since 1990, while the Lake Erie shoreline of Ohio has generally been below this criterion. The 10-year periods show that all regions were below the criteria in the 1980s, while all but the Lake Erie shoreline of Ohio have met the criteria during the 1990s.

This examination was based solely on counts of naturally produced, fledged young and did not include the 47 young that were either hacked (n=32) or fostered (n=15). The introduction of nestling eagles into the Lake Erie region that were not hatched from eggs laid by resident adults confounds the interpretation of the recovery of eagles within this region. The greatest occurrence of hacked/fostered young occurred between 1983/87, when 39 young were released. This represented an addition of 73% to the total number of naturally produced young during that same time period. The number of fledged young within the region increased 2-3 fold, from 15-20 fledged young annually to over 40 young fledged annually, five years after 1987. Since eagles mature over a five-year time span, it may be more than coincidence that this great increase occurred after the introduction of relatively clean eaglets into the region.

Table 2. Annual productivity (AP: number of fledglings/ occupied nest) and number of occupied nests (ON) of Bald Eagles nesting within 8.0 km of Lake Erie, 1980-1999. Comparisons are for the following regions: Ontario shoreline; Michigan shoreline; Lake Erie area of Ohio; and, Sandusky Bay area of Ohio. Values include only naturally produced young from Lake Erie nests and do not include the 47 young placed either in nests from zoos as fostered chicks (15) or hacked at Long Point, Ontario (32).

Year	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	X
Ontario AP	0.00	0.25	1.00	1.00	1.40	1.00	0.83	1.00	1.43	0.88	1.10	1.10	1.25	0.87	0.88	1.12	1.39	1.33	1.35	1.50	1.13
Ontario ON	3	4	5	5	5	6	6	6	7	8	10	10	12	15	16	17	18	18	20	14	2.75
Michigan* AP									1.00	0.00	0.00	0.00	0.50	0.67	0.50	1.25	1.25	1.25	1.50	1.75	1.03
Michigan ON									1	1	1	1	2	3	4	4	4	4	4	4	2.75
OH-L. Erie AP	0.67	1.00	1.67	1.33	0.67	0.33	1.25	1.00	0.60	0.60	0.80	0.00	1.17	1.14	0.29	1.29	0.86	1.63	0.80	0.30	0.85
OH-L. Erie ON	3	3	3	3	3	3	4	5	5	5	5	6	6	7	7	7	7	8	10	10	5.5
OH-Sandusky AP	0.50	1.00	1.00	0.67	0.00	0.00	0.33	0.80	1.17	0.67	0.67	0.44	2.00	1.22	1.44	1.20	1.09	1.33	1.07	1.67	1.10
OH-Sandusky ON	2	2	2	3	3	3	3	5	6	6	9	9	9	9	9	10	11	12	14	18	7.25
Lake Erie AP	0.38	0.67	1.20	1.00	0.82	0.58	0.85	0.94	1.11	0.70	0.84	0.58	1.41	1.00	0.86	1.18	1.20	1.38	1.17	1.33	1.05
Lake Erie ON	8	9	10	11	11	12	13	16	19	20	25	26	29	34	36	38	40	42	44	46	24.5

*Bald Eagles did not resume breeding on the MI shoreline of Lake Erie until 1988. Currently, Michigan only has four active breeding territories along Lake Erie.

Table 3. Mean annual productivity (AP: number of fledglings/ occupied nest) and mean number of occupied nests (ON) of Bald Eagles nesting within 8.0 km of Lake Erie, 1980-1999 for five-year intervals. Values include only naturally produced young from Lake Erie nests and do not include the 47 young placed either in nests from zoos as fostered chicks (15) or hacked at Long Point, Ontario (32).

		1980-84	1985-89	1990-94	1995-99
Ontario	AP	0.82	1.03	1.02	1.33
	ON	4.25	6.6	12.6	17.4
Michigan*	AP		0.50	0.45	1.40
	ON		1	2.2	4
OH-L. Erie	AP	1.07	0.77	0.68	0.93
	ON	3	4.4	6.2	8.4
OH-Sandusky	AP	0.58	0.70	1.16	1.31
	ON	2.4	4.6	9	13
Lake Erie	AP	0.84	0.85	0.95	1.25
	ON	9.8	16	30	42

*Michigan only had two nesting attempts in the period. Currently, Michigan only has four active breeding territories along Lake Erie.

Table 4. Mean annual productivity (AP: number of fledglings/ occupied nest) and mean number of occupied nests (ON) of Bald Eagles nesting within 8.0 km of Lake Erie, 1980-1999 for 10-year intervals. Values include only naturally produced young from Lake Erie nests and do not include the 47 young placed either in nests from zoos as fostered chicks (15) or hacked at Long Point, Ontario (32).

		1980-89*	1990-99*
Ontario	AP	0.95	1.20
	ON	5.43	15
Michigan	AP	0.50	1.06
	ON	1	3.1
OH-L. Erie	AP	0.89	0.82
	ON	3.7	7.3
OH-Sandusky	AP	0.66	1.25
	ON	3.5	11
Lake Erie	AP	0.84	1.13
	ON	12.9	36

*Michigan only had two nesting attempts in the period 1980-89.
**Michigan only has four active breeding territories along Lake Erie.

Deformities

From the early 1960s until 1998, a majority of nestling Bald Eagles in the Lake Erie area were banded, and measurements taken to determine age and sex. Since 1990, blood samples were collected from many of them to determine contaminant exposure. During the handling of these birds, a visual inspection for developmental deformities was made (Bowerman *et al.* 1994b; Bowerman *et al.* 1998). The rate of these deformities was compared to other published studies in avian populations.

From 1980/99, a total of 519 fledged young has been produced within 8.0 km of Lake Erie (unpublished data, P. Hunter, Ontario MNR; M. Shieldcastle, Ohio DNR; D. Best, USFWS, E. Lansing, Michigan). While not every single fledgling was banded, of those examined during that period, three nestlings with crossed bills have been found within these nests. All three of these nestlings were found in nests in Michigan, one each in 1993 and 1995 along the River Raisin and one in 1993 on the Wood Tick Peninsula. Based on feather plumage characteristics and (or) presence/absence of leg bands, at least one of the two adults at the River Raisin nest was replaced between 1993 and 1995 (Bowerman *et al.* 1998), suggesting that a site-specific factor (e.g., contaminants) rather than a parent-specific factor (e.g., genetic) caused the deformities. The feather plumage differences definitively showed turnover of adults based on replacement of a full adult (white head and white tail) with a near adult (some brown on head and (or) tail).

Rates for deformities are based on a number per 10,000 young (Fox *et al.* 1991). The rate of deformities in Bald Eagles from Lake Erie is 57.8 per 10,000 young. This figure is conservative, considering that not all 519 fledged young were examined and, if we include the fact that no fledglings were banded and examined for deformities in Ohio during 1998/99, this figure would actually be at least 64.9 per 10,000 young. This rate is much greater than the background rate of 0.5 per 10,000 young observed in avian populations (primarily songbirds and colonial waterbirds; Bowerman *et al.* 1994b). In comparison to other deformity rates in the Great Lakes region, this rate is greater than the 52.1 per 10,000 young rate observed in Double-crested Cormorants in Green Bay, which has the highest rate of deformities found in Great Lakes birds (Fox *et al.* 1991).

Based on results of studies on other colonial waterbirds and poultry, these teratogenic effects in nestling Bald Eagles are likely due to dioxin-like congeners of PCBs (Bowerman *et al.* 1994b; Giesy *et al.* 1994a; Ludwig *et al.* 1996; see section 7.7.1.2.1 for discussion of relationship between dioxin-like PCBs and deformities in birds).

Egg Concentrations

Unhatched eggs were collected from nests along the shorelines of Lake Erie in both Ontario and Ohio. Eggs were either collected after abandonment of the nest or were found within the nest lining during banding activities. Organochlorine pesticides, PCBs, and heavy metal concentrations were determined and related to a fresh, wet weight concentration. These concentrations were compared to a continental study to determine exceedence of the no and lowest observable effect concentrations (NOECs and LOECs, respectively).

A total of 32 unhatched eggs was collected from within 8.0 km of Lake Erie in Ohio from 1986/97 (unpublished data, D. Best, USFWS, E. Lansing). Criteria have previously been established for comparison of egg concentrations to reproductive endpoints such as eggshell thinning and embryonic mortality (Wiemeyer *et al.* 1984; Wiemeyer *et al.* 1993; Giesy *et al.* 1995). These NOEC criteria were:

- total PCBs 4.0 mg/kg;
- p,p'-DDE 3.5 mg/kg;
- dieldrin 0.1 mg/kg; all expressed as fresh, wet weight.

Of the 32 eggs analyzed, all exceeded the total PCBs criterion: 31 exceeded the dieldrin criterion and only seven exceeded the p,p'-DDE criterion.

A total of 12 eggs was analyzed from the Ontario portion of Lake Erie, six for the period 1974/80 and six for the period 1989/94. All 12 eggs exceeded the PCBs and dieldrin criteria: 10 exceeded the p,p'-DDE criterion (Donaldson *et al.* 1999).

One of the problems encountered with interpretation of the effects of environmental toxicants in a population of animals that is recovering and one that has few if any discernable characteristics among individuals, is the inability to discriminate among individuals from year to year. It has been observed, based on plumage characteristics and (or) presence/absence of leg bands, that a number of the Bald Eagles nesting along Lake Erie are replaced each year and that after the adults are replaced, the usual low reproduction within a breeding area increases for a number of years then declines (personal communication, M. Shieldcastle, Ohio MNR). Both the productivity analysis and the effects of environmental toxicants in eggs can be influenced by the replacement of adults within a population. This confounds our ability to truly track the influence of toxicants on this population. Therefore, we cannot solely rely on the productivity rate to indicate the health of this population. Since adults can be replaced within a breeding area and this may not be noticed, the concentrations of toxicants within failed eggs may give us a better view of the actual threat to the population than raw productivity data.

6.1.2 Use of Epidemiological Criteria to Weigh the Evidence of Reproductive and Deformity Impairment to Bald Eagles

Epidemiological criteria for causal inference were compiled by Fox (1991) to govern the exploration of cause-effect hypotheses related to ecosystem damage. These criteria were used to determine the likelihood of a causal relationship between reproductive and deformity impairments to Bald Eagles along the coast of Lake Erie and persistent toxic substances.

Data from this report as well as data contained within a previous review by Colborn (1991) were used to determine the association and strength of the relationships among various epidemiological criteria and reproductive impairment and deformities in Bald Eagles along Lake Erie (Table 5). It was determined that it is likely that eagle reproductive impairment was negatively influenced by PCBs, p,p'-DDE and possibly dieldrin. It was also determined that it is likely that deformities observed in Bald Eagles along the western basin of Lake Erie are associated with PCBs.

Summary

Up to and including the last five years, Bald Eagles nesting within 8.0 km of Lake Erie have impaired reproduction and developmental deformities associated with environmental toxicants in their prey from the Lake Erie ecosystem. Reproductive impairment is most clearly indicated by low fledging rates of the Ohio Lake Erie eagle population. The extremely high deformity rates demonstrate an impairment and other toxicological studies have shown these bill deformities are associated with dioxin-like chemicals, especially PCBs. Almost all eggs exceeded PCB and dieldrin NOECs and many eggs exceeded the DDE NOEC. Such chemical data, along with other studies, have clearly implicated organochlorines as important factors in the current reproductive problems experienced by Great Lakes Bald Eagles.

Determining the extent and magnitude of this impairment is confounded by the natural recovery of the Bald Eagle throughout North America and by the introduction of relatively uncontaminated nestlings through hacking and fostering projects along the Ontario and Ohio shorelines. The exact influence of this intervention cannot be measured due to the lack of a completely marked population of adults. However, there appears to have been a great increase in nesting success within the time period associated with sexual maturity of the greatest number of introduced young to this ecosystem. There also appears to be a relatively high turnover of adults along the Ohio and Michigan shorelines of Lake Erie, with an increased fecundity (i.e., production of young) for those nests after replacement of an older adult. Thus, it appears that immigrating adults and hacked/fostered chicks have bolstered the Lake Erie Bald Eagle population, potentially obscuring the reproductive effects of contaminants.

Table 5. Epidemiological criteria and strength of association to causal effects in Bald Eagle reproductive impairment and deformities in Lake Erie.

Criteria	Reproductive Impairment	Likely Chemicals	Deformities	Likely Chemicals
Time Order	Supports	DDE PCBs Dieldrin	Supports	PCBs
Strength of Association	Strong	DDE PCBs	Strong	PCBs
Specificity	Stronger for DDE than PCBs	DDE PCBs	Strong	PCBs
Consistency	High	DDE PCBs	High	PCBs
Coherence	Stronger for DDE than PCBs	DDE PCBs	Strong	PCBs
Plausibility	Both Biologically and Factually, Weakest for Dieldrin	DDE PCBs Dieldrin	Both Biologically and Factually	PCBs
Probability	Supports	DDE PCBs	Supports	PCBs

6.1.3 Colonial Waterbirds

Introduction

Fish-eating colonial waterbirds such as gulls, terns, herons, and cormorants are important predators and scavengers that feed near the top of the Great Lakes (Lake Erie) food web. This position makes them highly exposed to chemicals that bioaccumulate and biomagnify and they are sensitive to energetic changes in the food web that affect abundance of forage fish. Because these birds feed over a large area and are tied to the aquatic habitat, especially during the breeding season, they are excellent indicators of ecosystem health. They have been used to assess pollutant effects in the Great Lakes for more than 30 years. Although the relative sensitivity to particular contaminants varies by species, colonial waterbirds are sensitive to pollutants, as shown by effects on the biochemical, physiological, individual and population levels (Gilberston 1974; Gilbertson and Hale 1974; Gilbertson and Fox 1977; Fox *et al.* 1978; Ellenton *et al.* 1983; Moccia *et al.* 1986; Peakall and Fox 1987; Hoffman *et al.* 1987; Fox *et al.* 1988; Kubiak *et al.* 1989; Fox *et al.* 1991; Gilberston *et al.* 1991; Government of Canada 1991; Tillit *et al.* 1992; Hoffman *et al.* 1993; Ludwig *et al.* 1993a Rattner *et al.* 1993; Yauk and Quinn 1996; Grasman *et al.* 1998).

Whenever possible and appropriate, this section includes reproductive values (i.e., hatching and fledging success) that are considered to be normal or necessary to maintain stable or growing populations. Such reproductive values can serve as benchmarks for assessing reproductive impairments. Some data on population numbers are also included. However, it is important to note that while population-level problems are often caused by reproductive changes triggered by pollutants, food supply, etc..., reproductive effects do not immediately or always translate into population effects. If a population is near its carrying capacity, then there may not be enough resources (food, nesting habitat, etc...) for all young to survive to reproductive age. Hence, up to a point, a decrease in production of young due to contaminants may not affect adult population size because many young would have died anyway. However, if the population is below its carrying capacity, a decrease in production of young may slow the growth of the population back to the carrying capacity. Even if a population is at or near its carrying capacity, contaminant-induced reductions in reproductive success are a concern because if other stressors, natural or human-induced, reduce population size in the future, the reproductive effects of contaminants may slow or prevent population recovery.

Over the past 30 years, several outbreaks of deformities and embryonic mortality have been observed in fish-eating birds of the Great Lakes (Fox *et al.* 1991; Gilbertson *et al.* 1991; Grasman *et al.* 1998). Symptoms associated with these outbreaks include embryonic mortality, deformities (especially of the bill), hemorrhaging, gastroschisis (failure of the abdominal wall to close), and edema (fluid accumulation) of the head, neck, and abdomen that is frequently associated with embryonic death. This condition in wild birds has been named Great Lakes Embryo Mortality, Edema and Deformities Syndrome (GLEMEDS; Gilbertson *et al.* 1991). GLEMEDS is very similar to chick edema disease that was found in chickens accidentally fed PCB-contaminated food. Epidemiological and experimental evidence strongly indicates that

GLEMEDS is caused by the dioxin-like activity of PCBs (Gilbertson *et al.* 1991; Ludwig *et al.* 1996). Embryonic mortality is more important than deformities in terms of the number of birds affected and potential impacts on reproductive success and population dynamics. Accurate assessment of GLEMEDS requires examination of a large number of embryos and young birds, but such surveys have rarely been conducted for Lake Erie colonial waterbirds.

As the fields of biochemistry and cell biology have advanced, a number of new techniques have been applied to measuring effects of environmental contaminants. Such methods, which are often called biomarkers, have been used in studies of Great Lakes birds, especially Herring Gulls. These data for Lake Erie birds will be described here, when appropriate, because they indicate important mechanisms responsible for contaminant effects and provide a unique opportunity to monitor time trends in physiological impacts.

Trends in Populations of Fish-eating Waterbirds

While most species of fish-eating colonial waterbirds were affected, at least to some degree, by high concentrations of organochlorines in the 1960s and 1970s, population trends since the 1970s have varied by species (Austin *et al.* 1996; Blokpoel and Tessier 1996, 1998; Scharf 1998; Scharf and Shugart 1998). The nesting population of Double-crested Cormorants has increased dramatically on Lake Erie, as it has on the other Great Lakes (Weseloh *et al.* 1995). In 1976/77 there were 57 cormorant nests on one colony in Lake Erie; in 1989/90 there were 6,028 nests at five colonies (Table 6). The Lake Erie subpopulation comprised a relatively small proportion (8.6%) of the total Great Lakes population in 1989/91 (Table 6). The population of Black-crowned Night-herons decreased by 59% (Table 6). Decreases were seen at colonies both in Canadian and USA waters, where the number of nests decreased by 1,069 and 1,432, respectively. In Canadian waters, the largest active colony in 1977 was on the north end of Pelee Island; this colony was no longer active in 1990. The number of Great Egret nests has increased dramatically, from 221 at four colonies in 1976/77 to 1,568 nests at six colonies in 1989/91 (Table 6). The Great Egret population on Lake Erie comprised the majority (94.7%) of the Great Lakes population in 1989/91(Table 6). Of the Great Lakes other than Lake Erie, Great Egrets only nested at four colonies on Lake Huron in 1989/91 (75 nests) (Scharf 1998). The number of Great Blue Herons remained approximately the same during both censuses, and the Lake Erie population comprised approximately half of the Great Lakes population (Table 6). West Sister Island had the largest Great Egret and Great Blue Heron colony on Lake Erie in 1990 with 1,040 and 1,500 nests for each species, respectively.

For Ring-billed Gulls and Herring Gulls, the number of nests increased over 300% between the 1976/77 and 1989/91 (Table 6). In 1989/91, the largest Ring-billed colony on Lake Erie was at Port Colborne Mainland (43,590 nests). In 1989/91, the largest Herring Gull colonies were Middle Island (1,981 nests), Sandusky Turning Point (1,919 nests), Detroit-Edison (1,842 nests), and East Sister Island (1,556 nests). For both species, dramatic increases were seen in both Canadian and USA waters (Table 6). Great Black-backed Gulls and Caspian Terns were not found to be nesting on Lake Erie during the years when the censuses were conducted (Table 6). Numbers of Common Terns remained similar during both censuses (Table 6). The largest Common Tern colony in 1989/91 was on Port Colborne Breakwall (935 nests).

Table 6. Number of cormorant, heron, egret, gull, and island-nesting tern nests (number of colonies) in Canadian and USA waters of Lake Erie. Data presented from surveys conducted during official binational censuses (1976/77 and 1989/91). The percent change in nest numbers and percent of the Great Lakes population comprised by the Lake Erie subpopulations (during 1989/91) are given for each species. Data from: Blokpoel and Tessier 1993, 1996, 1997, 1998; Scharf 1998; Scharf and Shugart 1998.

	1976/1977			**1989/1991**			**% Change Between Surveys**	**% of Great Lakes Population (89/91)**
Species	**Canada**	**USA**	**Total**	**Canada**	**USA**	**Total**		
Double-crested Cormorant	57 (1)	0	57 (1)	1,956 (4)	4,072 (1)	6,028 (5)	10,475.4%	8.6
Black-crowned Night-Heron	1,220 (2)	3,000 (1)	4,220 (3)	151 (2)	1,568 (3)	1,719 (5)	-59.3%	36.4
Great Egret	21 (3)	200 (1)	221 (4)	143 (2)	1,425 (4)	1,568 (6)	609.5%	94.7
Great Blue Heron	76 (2)	2,538 (2)	2,614 (4)	368 (3)	2,546 (5)	2,914 (8)	11.5%	45.7
Ring-billed Gull	14,730 (4)	583 (2)	15,313 (6)	48,208 (4)	17,555 (5)	65,763 (9)	329.5%	11.4
Herring Gull	1,085 (6)	1,208 (6)	2,293 (12)	4,203 (8)	6,512 (40)	10,715 (48)	367.3%	12.3
Great Black-backed Gull	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.0%	0.0
Caspian Tern	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.0%	0.0
Common Tern	1,524 (4)	263 (1)	1,787 (5)	1,135 (2)	644 (5)	1,779(7)	-0.4%	17.7

Herring Gulls

Contaminant Trends

In the early 1970s, the Canadian Wildlife Service established a contaminant monitoring program using Herring Gull eggs collected from colonies throughout the Great Lakes, including Middle Island in the western basin and Port Colborne in the eastern basin of Lake Erie (Bishop *et al.* 1992; Pettit *et al.* 1994; Pekarik *et al.* 1998). This excellent data set allows an assessment of long-term trends in contaminants, particularly organochlorines. Like monitoring data using other species or media, the Herring Gull egg data show dramatic decreases in the concentrations of toxicologically important organochlorines such as PCBs and DDE in the late 1970s and early 1980s. Contaminant levels from this data set (and others) can be used to assess recent trends in contaminant concentrations and the magnitudes of any recent changes and to determine whether reproductive effects and deformities are expected at current exposure levels.

Several investigators have performed different statistical analyses on the Lake Erie Herring Gull egg data and have concluded that DDE concentrations show statistically significant declining trends (Pekarik and Weseloh 1998; Di Maio *et al.* 1999). PCB concentrations in Herring Gull eggs also continue to decline (Stow 1995; Pekarik and Weseloh 1998; Di Maio *et al.* 1999). Five-year averages were calculated for PCB 1254:1260 during the following five-year time periods: 1974/78, 1984/88, and 1994/98. The decrease in absolute values of PCBs is smaller between the 1980 and 1990 time periods, relative to the decreases between the 1970 and 1980 time periods (Table 7). Nonetheless, percent declines indicate similar rates of decline at Middle Island between 10-year intervals. At Port Colborne Lighthouse the rate of decline was faster from the 1980 to 1990 time period, compared to the rate of decline from the 1970 to 1980 time period (Table 7).

Table 7. Five-year averages for PCB 1254:1260 in Herring Gull eggs from Middle Island and Port Colborne Island, decrease in levels and percent decrease between time periods.

	Middle Island, Western Lake Erie			Port Colborne Lighthouse, Eastern Lake Erie		
Time Period	PCB 1254:1260 5-year mean (ug/g)	Decrease in levels (ug/g)	% decrease	PCB 1254:1260 5-year mean (ug/g)	Decrease in levels (ug/g)	% decrease
1974-1978	65.9			58		
1984-1988	40.5	25.4	38.5	26.3	31.7	54.7
1994-1998	26.9	13.6	33.6	9.5[a]	16.8	63.9

[a]: Five-year average (1994-1998) calculated using values from 1993-1998 (samples not analyzed in 1994 from this site).

Overall, levels of PCB 1254:1260 in Herring Gull eggs, were higher at colonies in the western basin of Lake Erie than those in the eastern basin (Table 7). Data presented in Bishop *et al.* 1992; Pettit *et al.* 1994; Pekarik *et al.* 1998 confirm this for colonies in Canadian waters, as do data for colonies in American waters (Grasman *et al.* 1996; K. Grasman, unpublished data). It is important to note that all reported concentrations for both the western and eastern basins are above the lowest observable effect concentration (LOEC) of 5.0 ug/g for embryonic deformities and egg lethality in Herring Gulls (Weseloh *et al.* 1991, as cited in Ludwig *et al.* 1993b).

Limited data are available for contaminant concentrations in the livers of breeding adult Herring Gulls from Lake Erie (Fox *et al.* 1998). Between 1980 and 1993, gulls from Middle Sister Island showed a 57% decline in liver PCBs and 59% decline in DDE. In 1993, the PCB 1254:1260 levels from Middle Sister Island (29.5 ug/g) were comparable to levels measured in 1991 at Saginaw Bay (31 ug/g) and Scotch Bonnet Island (28.3 ug/g) where levels were among the highest of all Great Lakes colonies sampled. Unlike PCBs, DDE levels in livers from Middle Sister Island were relatively low. For example, in 1993, concentrations from Middle Sister Island were 1.46 ug/g and in 1991, levels from Saginaw Bay and Scotch Bonnet Island were 6.5 ug/g and 7.4 ug/g, respectively. Between 1983 and 1991, gulls from Middle Island showed slight changes in liver PCB and DDE concentrations. PCB 1254:1260 levels decreased by 3.1% and DDE levels increased 2.3%. The PCB levels measured at Middle Island in 1991 (44 ug/g) were the highest of all Great Lakes colonies sampled in the early 1990s. Unlike PCBs, the DDE concentration at Middle Island was low (2.2 ug/g), relative to other Great Lakes sites.

In summary, data from Herring Gull eggs and adult livers show that Lake Erie, especially the western basin, still has elevated PCB concentrations. The levels remain within the range known to cause biological effects in Herring Gulls.

Reproductive Effects

A number of studies demonstrated that PCBs and DDE were associated with severe reproductive impairments and population declines in Herring Gulls throughout the Great Lakes during the 1960s and 1970s (Keith 1966; Gilbertson 1974; Gilbertson and Hale 1974; Gilbertson and Fox 1977; Gilman *et al.* 1977; Teeple 1977; Fox *et al.* 1978; Peakall *et al.* 1980; Grasman *et al.* 1998). However, effects during the 1970s were generally less severe in Lake Erie than in Lake Ontario. During the 1960s and 1970s, DDE was shown to cause eggshell thinning in a variety of upper trophic level birds around the world. In general, eggshell thinning of 16-20% is associated with population-level consequences. Several studies of Lake Erie Herring Gulls during the early 1970s suggested minimal eggshell thinning. Gilbertson 1974 and Gilman *et al.* 1977 reported eggshell thinning of 1.3-6.7% in eggs with DDE concentrations of approximately 7 ug/g. In the late 1970s, thinning varied between 1.9 and 11.5% across a number of Lake Erie colonies (Weseloh *et al.* 1990). Thus, even at the height of DDE contamination, eggshell thinning in Lake Erie Herring Gulls was generally below the level associated with widespread population problems. Even though there are no published reports of eggshell thickness in Lake Erie gulls during the 1990s, effects such as shell-thinning and other impacts on the endocrine system seem unlikely at current exposure levels (approximately 2 ug/g DDE in eggs).

There are many factors that influence embryonic mortality, therefore benchmark values for 'normal' rates of embryonic mortality in Herring Gulls vary considerably. For example, in one study embryonic mortality through three weeks of the four-week incubation period was found to be only 2% in Herring Gulls throughout the Atlantic seaboard (Kadlec and Drury 1968). Another study found an embryonic mortality rate of 28.6% through four weeks for gulls on Kent Island in the Bay of Fundy (Paynter 1949). During 1974, 37% of Herring Gull embryos at Port Colborne died during incubation; high concentrations of PCBs (65.8 ug/g Aroclor 1254:1260) were found in eggs (Gilman *et al.* 1977). In 1974, Herring Gull eggs were collected from colonies on Lakes Erie and Ontario and from a control site in New Brunswick, Canada. The eggs were incubated in the laboratory and rates of embryonic mortality were compared. Eggs from Lake Erie showed greater rates of embryonic mortality than those from the control site; nonetheless these rates were not as high as those seen in eggs collected from Lake Ontario (Gilbertson and Fox 1977). Recently, there have been few assessments of embryonic mortality for Lake Erie Herring Gulls. In 1992, gulls from Sandusky Bay experienced 29-34% embryonic mortality (Belant 1993; Belant *et al.* 1993). Current PCB concentrations in Lake Erie Herring Gull eggs, especially in the western basin (20 ug/g Aroclor 1254:1260), are sufficient to cause some embryonic mortality (LOEC 5.0 ug/g; see previous section on contaminant trends), although other factors also may influence incubation success.

For Herring Gulls, fledging rates of 0.8-1.0 young per nest are considered necessary for a stable population; 1.0-1.2 young per nest are associated with a growing population, assuming available resources (Government of Canada 1991). Gilbertson (1974) reported reproductive success to be less than 0.5 chicks fledged/nest for Herring Gulls nesting on Lake Erie in the early 1970s. Following significant reductions in organochlorine exposure during the mid-1970s, reproductive success increased to 0.95-2.17 chicks fledged/nest during the late 1970s through the mid-1980s, with most observations above the value of 1.0 for a growing population (Morris and Haymes 1977; Weseloh *et al.* 1990; Government of Canada 1991).

However, reproduction studies in the western basin (West Sister Island and Monroe, Michigan) during the 1990s suggest significant reproductive impairments, although the spatial and temporal distribution and the cause(s), of these reproductive problems remain to be determined (K. Grasman, unpublished data). During 1998 and 1999, excessive mortality two to four weeks after hatch caused almost complete reproductive failure on West Sister Island. Fledging rates were 0.19 chicks per nest in 1998 and 0.61 chicks per nest in 1999, which were also below the levels needed to maintain a stable population. Several hypotheses are currently being evaluated to explain this reproductive failure: PCB-induced wasting syndrome, infectious disease, cyanobacterial toxins (microcystins; Brittain *et al.* in press), or some combination of these factors. A potentially toxic concentration of microcystin liver toxin was found in one gull chick from the 1998 die-off (the only bird analyzed). No microcystin toxin was detected in two chicks found dead in 1999. During 1997, the fledging rate at Monroe was 1.76 chicks/nest, but many dead chicks were observed in 1998 and 1999, although fledging rates were not measured.

Deformities

No recent formal surveys of deformities in Herring Gulls have been published in the scientific literature. However, of the several hundred Herring Gulls examined in the course of other types of investigations, two deformed birds were found in western Lake Erie during the 1998 and 1999 breeding seasons (K. Grasman, unpublished data). During 1998, an adult male Herring Gull with a deformed bill was trapped on its nest at Monroe, Michigan, on the western shore of Lake Erie. However, the natal colony of this gull is unknown. In 1999, a three week old Herring Gull chick with a severely deformed bill was found on West Sister Island in the western basin of Lake Erie. These anecdotal observations do not allow the calculation of deformity rates for comparison to reference colonies. However, the concentrations of PCBs in the western basin are consistent with the presence of teratogenic deformities.

In early May 1997, a dead immature Great Black-backed Gull (*Larus marinus*) with a deformed bill (greatly extended lower bill) was found at Monroe on the western shore of Lake Erie (K. Grasman, unpublished data). The origin of this bird is unknown; the state of the carcass appeared to indicate that the bird died during the winter of 1996/97. Great Black-backed Gull eggs have been found to have higher levels of organochlorine contaminants than Herring Gull eggs collected at the same nesting colony (Pekarik *et al.* 1998). The higher trophic position occupied by Great Black-backed Gulls indicates that they are exposed to higher levels of organochlorine contaminants than Herring Gulls.

Biochemical and Physiological Effects

Several studies have demonstrated that organochlorine contaminants are associated with biochemical and physiological alterations in Herring Gulls from the western basin of Lake Erie and that the severity of these effects often ranks at, or close to, the top of all Great Lakes sites. In the laboratory, developmental and chronic exposure to PCBs and dioxins has been shown to cause immunosuppression, especially of T cell-mediated immunity. T lymphocytes are important for regulating immune responses (helper T cells) and attacking cells infected with viruses and bacteria (cytotoxic T cells). Using a skin test similar to a tuberculin test in humans, multiyear studies of Herring Gull and Caspian Tern chicks at many sites around the Great Lakes have shown suppression of T lymphocyte function at organochlorine contaminated sites (Grasman *et al.* 1996; Grasman, unpublished data). Statistically and biologically significant suppression of T cell-mediated immunity has been observed repeatedly at two colonies in the western basin: Monroe (1992, 1997) and West Sister Island (1998, 1999). These studies indicate compromised immune systems, potentially increasing susceptibility to infectious diseases.

In Great Lakes Herring Gulls, organochlorines also have been associated with enlarged thyroid glands and altered histological structure of these glands (microfollicular hyperplasia; Moccia *et al.* 1986; Fox *et al.* 1998). During the early 1980s, thyroids were most enlarged in adult gulls from Lake Erie compared to other sites within the Great Lakes. Decreases in organochlorine concentrations have been correlated with decreases in thyroid gland size in Lake Erie gulls, compared to thyroid gland size in the early 1980s. Nonetheless, thyroid glands in Lake Erie gulls are still enlarged compared to reference sites outside the Great Lakes (Fox *et al.* 1998). At Middle Sister Island, thyroid glands decreased in size between 1980 and 1993, nonetheless they were found to be the second largest of 11 Great Lakes sites sampled in the early 1990s. A detailed investigation of the effects of contaminants on thyroid physiology in Great Lakes Herring Gulls and laboratory-housed chickens has recently been initiated (A. McNabb, K. Grasman and G. Fox).

PCBs and dioxins can reduce stores of retinoids (various forms of vitamin A), which are essential for important physiological processes such as growth, development, vision, and immune function. During the early 1980s, adult Herring Gulls from the western basin of Lake Erie had the most severe depletion of retinoid stores in the liver compared to other Great Lakes sites (Moccia *et al.* 1986). Between the early 1980s and the early 1990s at West Sister Island and Middle Island in the western basin of Lake Erie retinoid stores in the liver improved but remained severely depleted compared to reference sites (Fox *et al.* 1998). Herring gull chicks at Monroe and other Great Lakes sites with high PCB concentrations had severely depleted concentrations of vitamin A in the blood in 1992 (Grasman *et al.* 1996).

Porphyrins are synthesized in the liver and incorporated into heme, which is an essential component of hemoglobin and some enzymes. PCBs and dioxins alter the biosynthetic pathway of heme in the liver, leading to the accumulation of highly carboxylated porphyrins that are normally intermediate (not final) products. Elevated liver porphyrin concentrations have been associated with environmental contaminants in adult Herring Gulls from the Great Lakes and have been used to monitor physiological responses to contaminants (Fox *et al.* 1998). Between

1974 and the early 1980s, there was no significant change in porphyrin concentrations in Lake Erie gulls. Between 1980 and 1993, there was no change in the elevated concentrations of porphyrins at Middle Sister Island in the western basin of Lake Erie. At Middle Island, concentrations of porphyrins increased 150% between 1983 and 1991, suggesting an increased bioavailability of porphyrogenic PCBs or an increase of an unidentified porphyrogenic chemical in Lake Erie.

These immunological and physiological impacts indicate biologically significant impacts of contaminants on the health of Lake Erie Herring Gulls. Such alterations may lead to consequences on the health of individual birds and potential impacts on reproduction and populations.

Double-crested Cormorant

Throughout the last 90 years, Double-crested Cormorant populations in the Great Lakes have changed dramatically in response to persecution by humans, environmental contaminants, and fluctuations in prey populations (Weseloh *et al.* 1995). Food supply is important both in the Great Lakes and the wintering areas (e.g., the expansion of fish farms in the southern USA during the last few decades). Cormorants appear to have colonized the Great Lakes during the early to mid-1900s, 1939 for Lake Erie (Weseloh *et al.* 1995). By the 1940s and 1950s, the total Great Lakes population was approximately 840-905 nesting pairs. Each of Lakes Superior, Huron, Ontario, and Michigan supported 100-400 nesting pairs, although the Lake Erie population was approximately 22 nesting pairs. However, during the 1960s and early 1970s the Great Lakes population crashed to approximately 89 nesting pairs, with the largest numbers of nests on Lakes Huron and Erie at approximately 40 nesting pairs each (Weseloh *et al.* 1995). Human persecution and food shortage cannot explain this population decline, which occurred after persecution by humans was reduced and in the presence of an abundant food supply (alewives).

Like other members of the order Pelicaniformes, cormorants are especially sensitive to DDE-induced eggshell thinning. In the USA and Canada, DDT use began in the mid-1940s and ended in the early 1970s. During the 1960s, Great Lakes cormorants experienced significant eggshell thinning associated with high DDE exposure. This eggshell thinning caused severely reduced hatching and fledging rates, resulting in the population decline noted above. Following the ban on DDT production in 1972, the degree of eggshell thinning decreased and the Great Lakes cormorant population exploded between the late 1970s and early 1990s. In Lake Erie, cormorant fledging success increased from approximately 0.5-0.6 young per nest during 1971/72 to 1.8-2.8 young per nest during 1979/84 (Weseloh *et al.* 1995). A fledging rate of 1-2 young per year is considered normal for cormorants (Government of Canada 1991). The Lake Erie cormorant population increased from 43 nesting pairs in 1970 to 2,380 nesting pairs in 1991 (Weseloh *et al.* 1995). In cormorant eggs from Middle and Big Chicken Islands in the western basin of Lake Erie, DDE concentrations fell from approximately 6.36 ug/g in the early 1970s to 2.40 ug/g in 1995 (Ryckman *et al.* 1998). At East Sister Island, eggshell thinning decreased from 9.5% in 1989 to 2.3% in 1995. Both chemical evidence and direct measurement of eggshell thinning show that, during the 1990s, DDE concentrations in Lake Erie cormorant eggs are below the critical effect level of 10 ug/g and eggshell thinning is below the effect level of 10% (Pearce *et al.* 1979).

While DDE-induced eggshell thinning clearly was an important factor in the decline of Great Lakes cormorant populations in the 1960s and early 1970s, other contaminants probably contributed to the problem. Cormorants at many Great Lakes colonies have exhibited embryonic mortality and deformities (GLEMEDS) and epidemiological and toxicological evidence strongly indicates that these deformities are associated with dioxin-like activity of PCBs (Gilbertson *et al.* 1991; Ludwig *et al.* 1995, 1996; see section on epidemiological evidence below). Unfortunately, the assessment of GLEMEDS in Lake Erie cormorants has been limited because they have nested almost exclusively in trees, precluding the examination of a large number of nests (usually thousands), which is essential for measuring deformity rates. The only published assessment of ground nests in Lake Erie showed no deformities in 720 chicks in the early 1980s (Fox *et al.* 1991). Embryonic mortality was not measured.

Although direct measurement of deformity rates and embryonic mortality in Lake Erie cormorants has been rare, exposure data from Lake Erie can be compared to exposure-response curves elsewhere in the Great Lakes to assess the potential occurrence of GLEMEDS in Lake Erie cormorants. The following PCB concentrations (Aroclor 1254:1260) have been reported for Lake Erie cormorant eggs: Big Chicken Island 45.5 ug/g in 1979, 38.7 ug/g in 1981 and 36.6 ug/g in 1983; East Sister Island 22.9 ug/g in 1989 and 20.8 ug/g in 1995 (data from Bishop *et al.* 1992; Pettit *et al.* 1994; Pekarik *et al.* 1998). Thus, these concentrations are all clearly above the LOEC of 3.5 ug/g for embryonic mortality in cormorants (Ludwig *et al.* 1993b). In fact, 20 ug/g ranks among or above the most contaminated Great Lakes colonies (primarily in Green Bay), leading to a prediction of 30-40% embryonic mortality and greater than 6% deformities in live eggs (Tillitt *et al.* 1992; Yamashita *et al.* 1993). During the mid-1990s, Lake Erie cormorant eggs had much higher (approximately 3X) PCB concentrations than other Canadian Great Lakes cormorants (Ryckman *et al.* 1998). This risk assessment strongly suggests the potential for significant reproductive and developmental effects in Lake Erie cormorants. Nevertheless, in Lake Erie, as well as elsewhere in the Great Lakes, these reproductive impairments have not been large enough to reduce population growth since the late 1970s, when DDE-induced eggshell thinning decreased in magnitude.

Common Tern

Organochlorine contaminants appear to have contributed to the decline of Common Tern populations in the Great Lakes during the 1970s (Weseloh *et al.* 1989). In Lake Erie, most published data are available for the Port Colborne colony in the eastern basin. During the early 1970s, fledging success at this colony was 0.78-0.94 young/nest/year, which was below the level of 1.1 young/pair/year that is necessary to maintain a stable population (Government of Canada 1991). Embryonic mortality ranged from 20 to 66% (Government of Canada 1991). Several deformed Common Tern chicks were observed in the early 1970s (Gilbertson *et al.* 1976). Organochlorine concentrations in eggs were quite high during the early 1970s: 37.8-78.8 ug/g PCBs (Aroclor 1260) and 5.4-10.9 ug/g DDE (data from Bishop *et al.* 1992). However, by 1981, contaminant concentrations had decreased to 4.8 ug/g PCBs and 0.95 ug/g DDE (Bishop *et al.*

1992). These PCB concentrations were below the LOEC of 7.60 ug/g for embryonic mortality in Common Terns (Bosveld and Van den Berg 1994). Eggshell thinning did not appear to be a problem at Port Colborne in 1981. This colony had the thickest eggshells compared to colonies in the Detroit River, Lake Huron, and Lake Ontario (Weseloh *et al.* 1989).

Recent data for Common Terns in the western basin indicate reproductive impairment primarily caused by predation (M. Shieldcastle, Ohio DNR, personal communication; Garcia 1997), but organochlorine concentrations in eggs are high enough to cause reproductive impairment. During the mid- to late 1990s, artificial nesting platforms were set out at the Ottawa National Wildlife Refuge (NWR) and Pipe Creek Wildlife Area (WA). Over six years (1994/99), fledging success has averaged 0.68 fledglings/pair at Ottawa NWR and 0.82 fledglings per pair at Pipe Creek WA. These averages are well below the level of 1.1 young/pair/year that is necessary to maintain a stable population (Government of Canada 1991). Success has been quite variable at both sites with complete failure (no fledglings) in some years and above 1.1 fledglings per pair in other years.

Predation by Great Horned Owls (*Bubo virginianus*), Herring Gulls, and fox snakes (*Elaphe vulpina*) appears to be the direct cause of the poor reproductive success. However, contributing effects of contaminants cannot be ruled out. Mean PCB concentrations in eggs ranged from 9.0-14.3 ug/g at Pipe Creek WA (1996 and 1995, respectively) and 12.0 ug/g at Ottawa NWR (1996). These concentrations are well above the LOEC of 7.60 ug/g for embryonic mortality in Common Terns (Bosveld and Van den Berg 1994). Mean DDE concentrations ranged from 1.2-1.4 ug/g at Pipe Creek WA (1996 and 1995, respectively) and 1.8 ug/g at Ottawa NWR (1996). Mean dieldrin concentrations ranged from 0.068-0.10 ug/g at Pipe Creek WA (1996 and 1995, respectively) and 0.113 ug/g at Ottawa NWR (1996). Contaminant-induced changes in incubation behavior of adults could contribute to the high predation rates observed for Common Terns in the western basin of Lake Erie. In Forster's Terns from Green Bay, high PCB contamination associated with parental inattentiveness during incubation, which led to decreased hatching success even in uncontaminated eggs placed in the nests of the contaminated adults (Kubiak *et al.* 1989). Similar effects on the incubation behavior of Lake Ontario Herring Gulls occurred during the mid-1970s (Fox *et al.* 1978; Peakall *et al.* 1980).

Caspian Tern

Caspian Terns do not regularly nest on Lake Erie. A small nesting colony (27 nests with eggs, 51 nests without eggs) was observed on Mohawk Island in the eastern basin during late June 1996 (D. V. Weseloh, personal communication). The Caspian Terns did not return to this site in 1997/99. Therefore, no field data are available concerning reproductive effects in Lake Erie. However, because the status of this species is of special concern to a number of agencies (e.g., considered threatened in Michigan) and because it has recently attempted to colonize Lake Erie, Caspian Terns have been included in the PCB risk assessment located in section 7.7. Studies of Caspian Terns elsewhere in the Great Lakes have demonstrated associations between organochlorines and immunological, physiological, reproductive and population-level effects (Ludwig *et al.* 1993b; Mora *et al.* 1993; Grasman *et al.* 1996, 1998).

Ecoepidemiological Analysis of Impairments

As described in section 6.1.2., a specific set of developmental problems and deformities in Great Lakes birds has been named Great Lakes Embryo Mortality, Edema and Deformities Syndrome (GLEMEDS; Gilbertson *et al.* 1991). For the purposes of this assessment, embryonic mortality associated with GLEMEDS qualifies as a reproductive impairment. There is strong evidence that GLEMEDS is observed consistently in multiple species and multiple locations within the Great Lakes and that these effects are associated with dioxin-like PCBs (Table 8; Gilbertson *et al.* 1991; Ludwig *et al.* 1995, 1996). The association between PCBs and rates of embryonic mortality and deformities is quite strong, as shown by the large differences in rates between highly contaminated and reference sites. These effects have been replicated in many locations, years, and species. With respect to specificity, embryonic mortality and deformities (crossed bill, club foot) are typical effects caused by dioxin-like chemicals in laboratory studies of birds. There are many factors that can cause embryonic mortality, so such mortality is not specific only to dioxin-like chemicals. However, the suite of deformities and edema seen in Great Lakes birds is quite specific to dioxin-like chemicals.

Following early evidence for a causal link between dioxin-like chemicals and GLEMEDS, similar effects were predicted and later verified at additional locations with high contamination by these chemicals. In terms of coherence, these effects are theoretically plausible and factually compatible with laboratory experiments demonstrating that dioxin-like chemicals cause embryonic mortality, edema, and deformities in birds (chick edema disease). Extensive surveys throughout the Great Lakes have provided strong statistical evidence for this causal association. For colonial waterbirds of Lake Erie, the statistical criterion is indeterminate because insufficient data are available from field surveys. However, incidental observations of a small number of crossed bills in Lake Erie gulls, out of several hundred birds examined, indicate the occurrence of deformities. Risk assessments for Herring Gulls, cormorants, and Caspian Terns strongly suggest that PCB concentrations in eggs from Lake Erie are above the lowest concentrations known to cause embryonic mortality and (or) deformities in each species. The time order criterion of the outbreak of GLEMEDS supports the hypothesis on causation by dioxin-like compounds because GLEMEDS symptoms were not reported in the time period before these chemicals were released in large quantities. The time order criterion for recovery is indeterminate because current concentrations of these chemicals are too high to allow for recovery.

At this time, there are too few data to complete an epidemiological assessment of the cause of high mortality rates in West Sister Island Herring Gull chicks.

Investigations in Herring Gulls and Caspian Terns of the Great Lakes have consistently shown associations between suppression of T cell-mediated immunity and organochlorine contaminants, especially PCBs (Grasman *et al.* 1996; K. Grasman, unpublished data). This impairment could lead to increased susceptibility to infectious diseases. The strength of this association is very strong; T cell immune function is drastically suppressed at highly contaminated sites. These immunosuppressive effects have been replicated in two species, in six years, and at many locations throughout the Great Lakes. Effects in Lake Erie have been shown in Herring Gulls from two sites in the western basin during multiple years of study. While many factors may cause immunosuppression, suppression of T cell-mediated immunity is one of the most commonly observed effects of exposure to dioxin-like chemicals. With respect to coherence, these effects are theoretically plausible and factually compatible with extensive laboratory experiments demonstrating that dioxin-like chemicals cause suppression of T cell-mediated immunity in birds and mammals. There is strong statistical support for the association between suppressed T cell-mediated immunity and organochlorines, especially PCBs. There are insufficient data to evaluate the time order criterion for the beginning of these immunosuppressive effects because immune function was not studied before the era of organochlorine pollution. The time order criterion for recovery is indeterminate because contaminant concentrations are still high enough to prevent recovery from this impairment. In Lake Erie, immunotoxic effects have been studied only in Herring Gulls, but similar effects are likely in other species of colonial waterbirds. Studies elsewhere in the Great Lakes have shown that Caspian Terns are more sensitive than Herring Gulls to these immunotoxic effects.

Several studies of biochemical impairments (decreased vitamin A, porphyrin accumulation in the liver and altered thyroid function) in Herring Gulls have shown that these endpoints can be used to monitor the health status of birds throughout space and time (Fox 1993; Grasman *et al.* 1996; 1998; Fox *et al.* 1998). In the Great Lakes, most of these biochemical effects are associated with organochlorines, especially PCBs. Associations between these biochemical effects and organochlorines are quite strong and have high statistical significance. These impairments have been replicated over time and at a wide variety of locations with significant contamination. Factors other than organochlorines can cause these effects, but organochlorines consistently produce these impairments. Hypotheses about causal linkages between organochlorines and these biochemical effects have allowed the verification of predictions about the occurrence of these effects in newly studied locations. In terms of coherence, organochlorines (especially PCBs) have been shown to cause decreased vitamin A, porphyria and altered thyroid status in laboratory animals. There are insufficient data to evaluate the time order criterion for the beginning of these biochemical effects because they were not studied before the era of organochlorine pollution. With respect to time order of recovery, biochemical impairments have improved (but not disappeared) as organochlorine concentrations in the Great Lakes have decreased. There is a large, long-term database on these biochemical impairments in Herring Gulls from several Lake Erie colonies.

Summary of Impairment Conclusions in Colonial Waterbirds

Available data indicate significant reproductive and physiological impairments and the presence of deformities in Herring Gulls from western Lake Erie. The causes of the reproductive impairment require further investigation. The deformities are most likely associated with dioxin-like PCBs. Many, but not necessarily all, of the physiological impacts are associated with PCB exposure. While the tree nesting habits of Lake Erie cormorants make reproductive assessments difficult, measurement of PCB concentrations in cormorant eggs suggests the strong probability of reduced hatching success and increased rates of deformities. Recent data for Common Terns in the western basin indicates reproductive impairment primarily caused by predation but organochlorine concentrations in eggs are high enough to contribute to reproductive impairment.

Caspian Terns attempted to colonize the eastern basin of Lake Erie in 1996 but have not returned in subsequent years. DDE-induced eggshell thinning does not appear to be important at current levels of contamination for any of the species examined.

Table 8. Epidemiological assessment impairments and their causes in colonial waterbirds of Lake Erie.

	Support for Presence of Impairment and Hypothesis about Contaminant Causation			
Criterion	Reproduction (Embryonic Mortality)	Deformity	Immunosuppression (T Lymphocyte Function)	Biochemical Effects (Vitamin A, Thyroid, Porphyria)
Time Order				
• beginning of impairment	+	+	I.D.	I.D.
• recovery	?	?	?	+
Strength of Association	+	+	+	+
Replication	+	+	+	+
Specificity				
• specific effect of hypothesized cause	+	+	+	+
• specific cause of an effect	?	+	?	?
Predictive Performance	+	+	+	?
Coherence	+	+	+	+
Probability	+	+	+	+
Species	Herring Gulls, cormorants, Common Terns, Caspian Terns (strongly suspected based on risk assessment)	Herring Gulls (documented); cormorants (strongly suspected based on risk assessment)	Herring Gulls	Herring Gulls
Likely Chemical(s)	PCBs	Dioxin-like PCBs	Organochlorines (including PCBs)	PCBs, possibly other porphyrogenic chemicals

+ Supports ? Indeterminate
- Detracts I.D. Insufficient data

6.1.4 Tree Swallows

Passerines (i.e., songbirds) bioaccumulate chlorinated hydrocarbons (Shaw 1984; Elliot *et al.* 1994; Hebert *et al.* 1994; Bishop *et al.* 1995) and trace metals (Krause 1989; Bishop *et al.* 1995) from contaminated ecosystems. In captive passerines dosed with p,p'-DDT or p,p'-DDE, effects induced included delayed ovulation, a decrease in weight and fertility of the eggs and reduction in chick survival (Jefferies 1967, 1971; Keith *et al.* 1993). However, Elliot *et al.* (1994) reported that despite p,p'-DDE concentrations of 80 ug/g in wild American Robin (*Turdus migratorius*) eggs from British Columbia, Canada, there were no significant effects on reproduction.

Tree Swallows (*Tachycineta bicolor*) are known to be exposed to PCBs and other organochlorine chemicals in the Great Lakes and St. Lawrence River wetlands (Bishop *et al.* 1995). Yet there are no laboratory studies examining the effects of PCBs on songbird reproduction. Therefore, the Canadian Wildlife Service examined chlorinated hydrocarbon residues, reproductive success, and sensitive biochemical indicators of contaminant exposure and effects (Boersma *et al.* 1986; Fox *et al.* 1988; Fox 1993) in Tree Swallows, a common resident of Canadian and USA wetlands.

Tree Swallows are small cavity-nesting, aerial insectivores that are used as biomonitors because they are readily attracted to nest boxes, making it easy to establish breeding colonies in shoreline wetland areas (Shaw 1984; Bishop *et al.* 1995; Custer *et al.* 1996; Yorks *et al.* 1996). Tree Swallows are appropriate monitors of local contaminant levels in wetlands on the Great Lakes because they feed on emergent invertebrates in close proximity to their nests (Bishop *et al.* 1995; Robertson *et al.* 1992). Despite their migratory habits, their eggs and chicks are indicative of local sediment contamination (Bishop *et al.* 1995, 1999).

In 1993/94, Tree Swallow eggs and 16-day old nestlings from seven wetlands in the Canadian Great Lakes-St. Lawrence River basin were analyzed. Six of these sites are designated as Areas of Concern (AOCs) due to persistent contamination, eutrophication, and (or) habitat loss. Two of these sites, Mud Creek and Wheatley Harbour, were in the Lake Erie watershed. Wye Marsh on Lake Huron was chosen as a reference site. Contaminant concentrations, biomarker levels and reproductive rates were compared among sites. Relationships among biochemical responses (biomarkers) and contaminant concentrations in these birds were examined. In 16-day old nestlings, the biomarkers measured in liver and kidney were vitamin A (retinol and retinyl palmitate), hepatic ethoxyresorufin-O-deethylase (EROD) and highly carboxylated porphyrins. Since interpretation of the effects of contaminants on vitamin A concentrations may be confounded by variable levels of vitamin A in the diet of wild animals, insects were collected, by ligature, from Tree Swallow nestlings from four sites in this study. Insects were analyzed for retinol and beta-carotene content.

There were marked differences in chlorinated hydrocarbon residues in eggs and nestlings among sites. However, there were no significant differences in hatching or fledging success. The maximum PCB concentration found was 11.1 ug/g in eggs from Akwesasne Reserve/St. Lawrence River, whereas the highest p,p'-DDE concentration of 2.57 ug/g was found in eggs from Mud Creek, in the Lake Erie watershed. Tree Swallow eggs from Wheatley Harbour (Lake

Erie) were contaminated with PCBs at 0.64 ug/g and with DDE at 0.10 ug/g, similar to concentrations detected in eggs from Toronto Harbour and Hamilton Harbour in Lake Ontario. This is likely due to the known PCB contamination in Wheatley Harbour from the Omstead Fish Processing Plant. The source of the DDE in Tree Swallows at Mud Creek is currently unknown, although the general area was heavily sprayed with DDT in the past to combat pests of tobacco plants. Concentrations of other organochlorine pesticides and chlorobenzenes were low and not variable among sites.

Significant differences in hepatic EROD activity, retinol, and retinyl palmitate and uroporphyrin in nestling birds existed among sites. Although there were no significant differences, liver retinyl palmitate was quite low (<50 ug/g) in samples from Mud Creek compared to control sites and other areas on the Great Lakes but were similar to samples from two sites highly contaminated with PCBs in the St. Lawrence River. EROD activity was highest and retinol and retinyl palmitate were lowest at Cornwall Island, St. Lawrence River, while porphyrins were highest at Toronto and Hamilton Harbours, Lake Ontario. EROD activity was significantly and negatively correlated with nestling hepatic retinol concentrations whereas uroporphyrin concentrations were positively correlated with PCB congener #118 in nestlings. In contrast, there was little difference in vitamin A concentrations in diet samples among sites, suggesting that reduced vitamin A in swallows was not associated with lack of beta-carotene in food sources. At Mud Creek and Wheatley Harbour in Lake Erie, liver retinol concentrations and EROD activity were similar to the reference site (Wye Marsh) on Lake Huron. Depressed hepatic vitamin A and elevated porphyrin concentrations are strongly associated with high organochlorine exposure in Tree Swallows and indicate biochemical effects that may influence the health of the birds. However, there does not appear to be significant reproductive impairment in the Lake Erie Tree Swallows. While Tree Swallows are good indicators of exposure to contaminants via aquatic insects, they tend to be resistant to the reproductive impacts (measured in terms of embryonic survival and fledging success) of organochlorine exposure. While other species of insectivorous birds in Lake Erie may be more sensitive to the reproductive effects of contaminants, no research has been done for verification.

6.2 Mammals

6.2.1 River Otter

Although little Canadian information is available, the river otter was likely present historically in suitable habitat around Lake Erie reproducing successfully in what would then have been uncontaminated habitats. In the USA, river otters were extirpated from the Lake Erie watershed by 1900 in Ohio, Michigan, Pennsylvania, and New York, probably due both to extensive habitat loss and over-trapping (Chris Dwyer, Ohio DNR, personal communication). A similar situation probably also occurred in Ontario. Reintroductions of otter, which have been ongoing since the late 1970s in Pennsylvania, occurred between 1986 and 1992 in Ohio and beginning in 1996 in New York State. However, locations for reintroductions have been based on appropriate habitat and water quality criteria and hence have occurred primarily inland from the Lake Erie watershed. Between 1986 and 1988, animals were released into the Grand River in Ohio, which drains into Lake Erie. Unfortunately, systematic monitoring of otter populations in any of these states is not in place and population assessments are based primarily on voluntary reports of observations. Nevertheless, populations of Pennsylvania river otter are judged now to be stable or increasing, with new introductions being made in watersheds as they achieve water quality standards; however, none are associated with Lake Erie (Tom Hardiski, Pennsylvania DNR, personal communication). The status of the Ohio population is less sure, with only an estimated 30 observations being made per year statewide since 1992 (Chris Dwyer, Ohio DNR, personal communication). Sightings of single animals are occasionally made in Lake Erie wetlands associated with the Grand River, whereas family group sightings are frequently made at inland locations.

The extent to which contaminants have impaired the re-colonization of the Lake Erie basin by otters is unknown. Presently little toxicological work has been conducted on river otter in North America. In studies where body contaminant levels were compared with mink from the same area, river otter had higher contaminant levels (Henny *et al.* 1996), reflecting their more exclusively aquatic diet, higher trophic level, and greater lifespan (Errington 1954; Hamilton 1959; Wren 1991; Dunstone 1993). In first year male river otter in the Lower Columbia River in Oregon, PCB (Aroclor 1260) liver concentrations were inversely correlated with testes size and baculum (penis bone) length and weight (Henny *et al.* 1996). However, juvenile otters collected in British Columbia, within the same range of PCB contamination, failed to show this correlation (Harding *et al.* 1999). Nevertheless, size of male reproductive organs from a pristine site in Oregon was significantly larger than those from either of the other sites (Harding *et al.* 1999). Laboratory studies have shown that the non-ortho PCBs are the congeners responsible for adverse reproductive effects in mink (Aulerich *et al.* 1985; Brunstrom *et al.* 1991; Kihlstrom *et al.* 1992). River otters, like mink, have been shown to selectively retain these congeners (Bergman *et al.* 1992; Kihlstrom *et al.* 1992; Tillitt *et al.* 1996; Leonards *et al.* 1997) and it is suggested that they may be at least as sensitive to PCBs as are mink (Leonards *et al.* 1998).

In Europe, otter have declined over the last forty years and PCB contamination has been implicated. Clean-up efforts over the last decade have resulted in reductions of PCB residues of 7-8% per year in otter tissues (Mason 1998). Although populations are recovering and reintroductions occurring in some areas (Mateo *et al.* 1999), Kruuk and Conroy (1996) suggest that the unusually slow recovery of British otters may be due to food shortages and the PCBs may have been only partially responsible for initial declines.

6.2.2 Mink

Population Trends

Existing information on mink populations in the Lake Erie basin is obtained from fur-trapping statistics generally collected on a county or township basis. Trapping data provide our only estimates of mink population abundance, but factors such as trapping effort and correlation with muskrat abundance confound the analyses of trapping data. Some assessments of numbers of trapped mink suggest lower mink populations along Lake Erie, but other assessments show little difference from inland areas. Fur prices affect trapper effort, which may bias the assessment of mink abundance. Many mink are trapped inadvertently during muskrat trapping; so reduced muskrat trapping effort can reduce the number of mink trapped. Ohio fur buyer records (Tori 1995) indicated low statewide mink submissions in the late 1960s, which climbed steadily into the early 1980s. Throughout this period, muskrat numbers were high and relatively constant. Mink numbers have again declined since the mid-1980s and by 1995 were similar to the low numbers of the 1960s. During this period, however, muskrat submissions also dropped drastically to 25% of earlier numbers, suggesting harvest of the two species was related. In contrast, in Pennsylvania and New York State, there is a general impression on a statewide basis that, while muskrat populations have been decreasing due to alterations of the agricultural habitat to which they had become adapted; mink have been gradually increasing since the 1960s (Tom Hardiski, Pennsylvania DNR, personal communication; Mike Ermer, New York State DEC, personal communication).

Determining population trends within the Lake Erie basin from region-wide harvest data is difficult, but has been attempted in two regions. Wren (1991) reported that mink harvest in Ohio counties adjacent to Lake Erie was less than half that of counties immediately inland throughout the 1980s. However, use of an index of mink trapped relative to trapper effort from 1990/94 showed greater returns in shoreline counties in 2/5 years and lower returns in 3/5 years, with no trends over time (Tori 1995). In Ontario, Glooshenko *et al.* (1990) found that mink harvest between 1970 and 1985 in townships bordering Lakes Erie and Ontario and the St. Lawrence River (considered to be high PCB areas) was lower than that in adjacent inland townships. Welch (1992) however contended that within a coastal township few if any animals might actually be taken from shoreline areas, but instead from waterways within the watershed, which would be removed from contaminants within Lake Erie. In a detailed trapper survey of mink trapped in townships bordering the north shore of Lake Erie, Welch (1992) found that, while total harvests decreased dramatically from 1987 (>500 animals) to 1991 (50 animals), the proportion of mink trapped within one mile of the Lake Erie shore remained similar (17%) throughout the study period (1985/91), indicating no differences in trend with proximity to the more relevant high PCB exposure areas within shoreline townships.

Field Toxicology Studies

Results of field studies on the ecotoxicology and reproduction of wild mink relative to environmental contamination are not available at present. Such studies are very difficult to carry out because: 1) mink are highly sensitive to disturbance in the vicinity of their denning sites; 2) mink are generally trap-shy and therefore, difficult to catch live; and 3) if trapped for individual marking, are often very difficult to re-trap.

Harding *et al.* (1999) related morphology of reproductive organs to PCB levels in mink trapped in British Columbia and found a significant negative correlation of baculum (penis bone) length to liver PCB concentration in juvenile animals at concentrations between 0.02 and 0.18 mg/kg wet weight. Recent work on Lake Erie mink however indicates that this relationship is non-significant in juvenile mink and that in adult mink, a significant positive relationship occurs at

PCB levels between 0.1 and 1.5 mg/kg (Martin *et al.* 2001). Implications of reductions in baculum size for reproduction are unknown. Very little laboratory work has assessed effects on males. However, overall rates of developmental abnormalities in reproductive, digestive and renal systems in the western population were relatively high at 10%, whereas very few abnormalities were noted in the Lake Erie population.

Laboratory Toxicity Experiments

Laboratory experiments indicate that PCBs can be directly lethal to adult mink at fairly low levels of 4-5 mg/kg wet weight in liver (Aulerich and Ringer 1977) and that they are more sensitive than other species of mustelid (Bleavins *et al.* 1980), although river otter may be similar to mink in their sensitivity to PCBs (Leonards *et al.* 1998). More importantly, they show reproductive and immune effects at much lower levels of PCB exposure. The mechanism for reproductive effects appears to involve function impairment of uterine steroid receptors, rather than changes in concentrations of circulating hormones (Patnode and Curtis 1994; Shipp *et al.* 1998).

Many studies examining effects of PCBs on mink have used contaminated Great Lakes fish with known contaminant concentrations, rather than pure chemicals or technical mixtures. Since the proportions of the various congeners in fish are different from those of the compound as it was released into the environment, this method of exposure was deemed most appropriate. Female mink fed a diet of Saginaw Bay carp containing 2.56 mg/kg PCBs produced no viable kits (Heaton *et al.* 1995). Deformities (subcutaneous edema and mandibular shortening) were observed in 2/16 stillborn kits in six litters. Liver PCB concentrations in these females were 6.3 mg/kg wet weight (Tillitt *et al.* 1996). In kits of females fed diets containing 0.72 and 1.53 mg/kg PCBs, there were significant reductions in survival rate (by approximately 65% for both groups) and mean body weight (30% less in both groups) compared to controls, by three weeks of age. In a group fed diets containing 2.56 mg/kg PCBs, there was total kit mortality by three weeks. A LOEC of 0.72 mg/kg in the diet, equaling 0.134 mg PCB/day, was determined (Heaton *et al.* 1995). Corresponding PCB concentrations in the liver of the females in this group was 2.2 mg/kg wet weight (Tillitt *et al.* 1996). Earlier studies yielded similar reproductive results although the female PCB body burden is seldom reported. Hornshaw *et al.* (1983) reported female mink fed diets containing 1.5 mg/kg PCBs suffered complete reproductive failure, as did mink fed 1.64 mg Aroclor 1254/day (Kihlstrom *et al.* 1992). Wren (1987) found reduced growth rate in kits nursed by mothers consuming diets containing 1.0 mg/kg PCBs. Kihlstrom *et al.* (1992) calculated that 50% and 100% decreases in litter size occurred at muscle concentrations of 75 and 180 mg/kg PCB, lipid weight (approximately 3.75 and 13 mg/kg wet weight, respectively) in the mother. Leonards *et al.* (1995) proposed a critical whole body residue level for 50% reduction in litter size (EC50) of 1.2 mg/kg total PCBs (wet weight) in the mother, based on published data from the literature. In a more recent study however, Restum *et al.* (1998) found significant reductions in kit body weight at three weeks of age in first litters where the mothers were fed a diet containing as low as 0.25 mg/kg PCBs (wet weight) during pregnancy and lactation and reduced birth weight of kits in second litters of females fed 0.25 mg/kg continuously. There was also significantly decreased survivability in second litters of females fed 0.5 and 1.0 mg/kg PCBs in the diet, continuously. Therefore, a LOEL of 0.25 mg/kg in the diet was calculated and corresponded with a maternal liver PCB concentration of 0.98 mg/kg (wet weight). Almost complete failure in kit survival (diet of 1.0 mg/kg PCBs) corresponded to maternal liver PCB concentrations of approximately 1.5 mg/kg.

Exposure to halogenated aromatic hydrocarbons such as PCBs, particularly prenatally, are known to cause immunosuppression in mammals (Vos and Moore 1974). Brunstrom *et al.* (1991) found a reduction in the number of thymocytes in mink exposed *in utero* to mono- and non-ortho PCB congeners. Increases in spleen weight of both female and kits with increasing exposure to PCBs (Heaton *et al.* 1995; Restum *et al.* 1998) also suggest impacts on immune function at levels similar to those associated with reproductive effects.

Tissue Residues – Field Collections

Two collections of wild mink from the north shore of Lake Erie have been made in the last 25 years for the analysis of PCB and other chlorinated hydrocarbons. In 1979, Proulx *et al.* (1987) found PCB (Aroclor 1254:1260) concentrations greatest in Mersea township (Point Pelee, Ontario) in the west basin (mean = 29 mg/kg lipid basis [1.32 mg/kg wet weight], whole body) and Dunn-Rainham township at the mouth of the Grand River, Ontario, in the east basin (mean = 26 mg/kg lipid basis [1.71 mg/kg wet weight], whole body). In 1988/89 (Haffner *et al.* 1998), mink PCB concentrations were highest at Mersea (23 mg/kg lipid basis [1.79 mg/kg wet weight], liver). Mink were not collected from Dunn-Rainham Township during that study. Initial results of a recent collection (Martin *et al.* 2001) indicate that, on a lipid weight basis, mean Aroclor 1254:1260 levels in Dunn-Rainham mink have increased to 50 mg/kg (0.879 mg/kg wet weight). Near Long Point Provincial Park, Ontario, PCB levels in mink increased from 10.7 to 30.9 mg/kg lipid weight between 1979 and 2000. No recent data are available as yet for Mersea Township.

Conclusions: Relating Environmental Residue Levels to Laboratory Effect Levels

Given the mean concentration of mink trapped from Mersea township in the late 1980s was 1.79 mg/kg wet weight in liver and that maternal mink in the lowest observable adverse effect group (determined by Restum *et al.* 1998, above) had a mean liver concentration of 0.98 mg/kg in liver, it is likely that some degree of reproductive impairment is occurring in Lake Erie mink. Of 34 mink analyzed in 2000, three (8.8%) had liver PCB levels exceeding this LOAEL. All three were males. An additional three male mink had liver PCB levels approaching the LOAEL, between 0.5 and 0.98 mg/kg wet weight (Martin *et al.* 2001). Unfortunately, no laboratory studies have assessed reproductive effects and concentrations in male mink, so the consequences of these levels in wild mink are unknown. In a small sample of wild mink, females (n = 4) had significantly lower liver PCB concentrations than did males (n = 14) (Martin *et al.* 2001), a fact that may be the consequence of females clearing themselves of their burden when bearing young and lactating. Age of female animals was unknown. Reduced burdens in females may also be a result of differential in diets between the sexes, and could have positive implications for effects on reproduction.

The relatively high rates of reproductive and other abnormalities (10%) in wild juvenile mink trapped in British Columbia, corresponding to low levels of PCB contamination (< 0.20 mg/kg wet weight liver) supports the conclusion that animals in Lake Erie, probably at higher levels of contamination, are also experiencing at least these rates of deformity; however, deformities were not apparent in mink collected recently.

PCB concentrations measured in mink prey in Lake Erie provide evidence that foraging mink are exposed to contaminant levels associated with reproductive disturbance. In the western basin in 1991, small-mouth bass contained muscle PCB concentrations of 3.8-11.2 mg/kg wet weight (Koslowski *et al.* 1994). Values for other potential mink food items in Lake Erie include spiny softshell turtle eggs at Rondeau Park (5.68 mg/kg Aroclor 1254:1260) and Pelee Island water snake liver (6.58 mg/kg Aroclor 1254:1260) (Bishop and Gendron 1998). The authors of this assessment are unaware of any contaminant values for Lake Erie muskrats, which are assumed to be extremely important in the diet of marsh-inhabiting mink. Muskrat concentrations are likely to be much lower than fish, given their primarily herbivorous diet. Nevertheless, given the opportunistic nature of mink foraging habits (Dunstone 1993), it is likely that fish and turtles, which contain PCB concentrations far in excess of the LOEC of 0.25 mg/kg, comprise a significant portion of their diet over the year.

6.2.3 Use of Epidemiological Criteria to Weigh the Evidence of Reproductive and Deformity Impairment to Lake Erie Mink and Otter

An ecoepidemiological analysis supports the presence of reproductive impairments in mink, with a causal link to PCBs. A similar impairment and cause are suggested for otter, although the evidence is less clear than for mink.

Time-Order Relationships

Otter populations in the Lake Erie basin were decimated by over-harvesting and habitat destruction long before the production of PCBs and other chlorinated hydrocarbons. Therefore, the outcome (population reduction) preceded the proposed cause (PCB contamination). Reintroductions into relatively uncontaminated sites near Lake Erie have unconfirmed results. In Europe however, otter populations plummeted following habitat contamination and have slowly rebounded as cleanup is accomplished, supporting the hypothesis. Mink harvest data have fluctuated considerably over time but there is no consistent trend relative to the onset of PCB contamination.

Strength of Association

Examination of mink harvest data over the same time period in areas of high and low PCB exposure in Ontario and Ohio generally, but weakly, support the hypothesis. There is insufficient information on otters to make an association.

Specificity

Laboratory studies show high specificity of effects in mink in response to chemical exposure, although this cannot yet be extrapolated to wild populations. However, the specificity of the cause to the effect of reduced harvest in wild populations is very low, as many other factors can be involved, such as trapper effort, disease, and fur prices.

Consistency

In laboratory studies with mink, effects of reproductive impairment are consistently shown to be produced by PCB exposure. In the absence of laboratory studies on otters, studies of wild European populations provide relatively consistent support for the hypothesis.

Coherence

Theoretical. Based on laboratory studies with mustelids, it is highly plausible that chemical contamination in Lake Erie could be adversely affecting mink and otter populations.

Biological. Laboratory research indicates the hypothesis of chemical disruption of mustelid populations in Lake Erie is biologically coherent. More and better data are needed on population trends and on actual exposure via tissue residue analysis.

Factual. In the Lake Erie basin the data on mink population trends in high and low risk areas are somewhat contradictory and of intermediate compatibility with the hypothesis. There is not enough information on Lake Erie otter populations to support or refute.

Statistical. No statistical attempt at drawing a relationship between the cause and effect in Lake Erie mustelids has been made.

Conclusions

Several of the epidemiological criteria support the hypothesis that PCB contamination in the Lake Erie basin may be harmfully affecting mink populations. Laboratory studies provide unquestionable evidence that mink are extremely sensitive to this contaminant and the few existing data on Lake Erie mink tissue residues indicate wild mink are being exposed to potentially harmful levels of PCBs. However, the lack of unbiased, systematic population data makes extrapolation of these finding to wild mink uncertain. For otter, there are no data to logically support or refute the hypothesis of PCB impacts on populations, although evidence from other geographic locations indicates a cause and effect relationship.

Table 9. Epidemiological criteria weighing evidence of impairments of Lake Erie basin mink and otter.

	Support for hypothesis of contaminant impairment of reproduction	
Criteria	**Mink**	**Otter**
Time Order		
beginning of impairment	I.D.	-
recovery	I.D.	+
Strength of association	**weak**	**?**
Specificity		
specific effect of hypothesized cause	+ (in lab)	?
specific cause of an effect	+ (in lab)	?
Consistency	+	+
Coherence		
plausible	+	+
biological	+	+
factual	+	+
Likely Chemical	**PCBs**	**PCBs**

+ Supports ? Indeterminate - Detracts I.D. Insufficient data

6.3 Reptiles

6.3.1 Snapping Turtles

In the early 1980s, snapping turtle eggs from Rondeau Provincial Park and Big Creek Marsh (at Long Point, north shore of Lake Erie) contained PCBs and some organochlorine pesticides (Table 10; Bishop *et al.* 1996). Among the organochlorine pesticides, concentrations of p,p'-DDE were detected at the highest concentrations.

More recently, snapping turtle eggs were collected at six sites in the Lake Erie basin in Ohio in 1997 (Dabrowska *et al.* 1999). Concentrations in those eggs ranged from 0.183 to 3.683 ug/g wet weight. Concentrations of 2-3 ug/g PCBs in eggs are equal to those from Hamilton Harbour (Bishop *et al.* 1996, 1998b) where summed rates of deformities in hatchlings plus rates of unhatched eggs are significantly elevated above those at a long-term reference population in Algonquin Provincial Park, Ontario.

Assessing Reproductive Impairments

The following rates are based on artificial incubation of eggs. At Big Creek Marsh in Lake Erie

(Long Point, Ontario) during 1986, the average rate of unhatched eggs was 11% and the deformed hatchling rate was 0%. In 1989, the rate of unhatched eggs was 2.6% and the rate of deformed hatchlings was 5.3% (Bishop *et al.* 1991; 1998b). At Rondeau Provincial Park, Lake Erie, the incidence of unhatched eggs was 5.2% and the rate of deformed hatchlings was 7.1% in 1989 (Bishop *et al.* 1991; 1998b). In some years, the incidences of those anomalies in the Lake Erie sites exceeded those in an inland reference site, Algonquin Provincial Park, but the differences were not statistically significant (Bishop *et al.* 1998b). Larger sample sizes (i.e., more comparable to the surveys of thousands of Great Lakes colonial waterbirds) might increase statistical power for detecting deformities in snapping turtles.

In the 1980s and 1990s, a sexually dimorphic trait of adult snapping turtles was measured in populations from two reference sites in central Ontario (Algonquin Provincial Park, sampled in 1987/91; and Jack Lake, sampled in 1995) and in three contaminant-exposed sites: Coot's Paradise in Hamilton Harbour (sampled in 1994/95) and Lynde Creek in Lake Ontario (sampled in 1995) and Big Creek Marsh at Long Point, Lake Erie (sampled in 1986/87). In the Lake Ontario and the Lake Erie populations, the degree of sexual dimorphism in male snapping turtles was statistically less pronounced and more female-like compared to reference sites (de Solla *et al.* 1998).

Potential Linkages to Human Health

The Lake Erie LaMP Human Health Subcommittee (HHSC) has been charged with assessing the threat to human health from critical pollutants and other contaminants of concern. Their detailed background assessment has not yet been through LaMP review. Therefore, what is known about this issue in terms of levels of toxic contaminants in turtle flesh is presented here as a basis for further work on the part of the LaMP HHSC.

In 1988/89, concentrations of chlorinated hydrocarbons were measured in Ontario snapping turtle muscle to determine if these levels exceeded fish consumption guidelines. Samples were collected at Walpole Island and Stoney Point on Lake St. Clair, Turkey Creek which flows into the Detroit River, Hillman Marsh, Turkey Point, and Long Point on Lake Erie (Hebert *et al.* 1993). PCBs, DDT, mirex, and octachlorosytrene (OCS) were measured in turtle muscle, which is the tissue used in soups for human consumption. Only concentrations of OCS exceeded guidelines for consumption among the turtles sampled. OCS levels in muscle of larger turtles from Walpole Island and Stoney Point exceeded the 2 ng/g wet weight guideline set by New York State for consumption of edible portions of fish (Hebert *et al.* 1993). New York guidelines were used, as Ontario did not have guidelines for OCS in fish fillets at that time.

In 1997, concentrations of organochlorine pesticides, PCBs, mercury, lead and cadmium were measured in Ohio snapping turtle muscle, liver, and body fat at the six locations referenced in Table 10. Mercury, lead and PCB-1260 were the most frequently detected chemicals in Ohio snapping turtle muscle. Specifically, mercury was detected 59 times at concentrations ranging from 16-391 ug/kg wet weight, PCB 1260 was detected 12 times at concentrations ranging from 56-250 ug/kg wet weight, and lead was detected seven times at concentrations ranging from 129-

1060 ug/kg wet weight (Ohio EPA, unpublished). No analysis of these concentrations in terms of potential impacts to human consumers of turtle muscle has been conducted to date.

Table 10. Contaminant concentrations in Lake Erie basin snapping turtle eggs (calculated from Bishop *et al.* 1996; Dabrowska *et al.* 1999). (N=clutches sampled)

Location	Years Sampled	Arithmetic mean summed congener-specific PCB concentrations (ug/g wet wt.)	Arithmetic mean p,p'-DDE concentrations (ug/g wet wt.)
Big Creek Marsh (Long Point, Ontario)	1981 (N=5) 1989 (N=7)	1 0.34	0.01 0.04
Rondeau Provincial Park	1984 (N=5) 1989 (N=7)	1.1 0.625	0.04 0.04
River mile 4.5, Ottawa R.	1997 (N=3)	3.683	N/A
River mile 1.0, Maumee R.	1997 (N=3)	2.519	N/A
River mile 1.98, Black R.	1997 N=1)	0.873	N/A
River mile 1.6, Ashtabula R.	1997 (N=2)	1.08	N/A
Ottawa National Wildlife Refuge+	1997 (N=4)	0.352	N/A
River mile 61.4, Lake Rockwell +	1997 (N=4)	0.183	N/A
+ Reference Site			

6.3.2 Eastern Spiny Softshell Turtle

Under the province's *Fish and Wildlife Conservation Act*, the eastern spiny softshell turtle is a threatened species in Ontario. Nationally in Canada, this species is also designated threatened. Consequently, reproductive impairment in this species is of interest to those involved in implementing the recovery plan for this species. Hatching success of eastern spiny softshell eggs incubated in natural nests was assessed in Ontario in 1996 at Long Point, Thames River, and Rondeau Provincial Park. Eggs from Long Point did the poorest, followed by Thames River and Rondeau nests. At Long Point, hatching success was 45.4% (N=30 clutches); at the Thames River, it was 67.1% (N=13 clutches) and at Rondeau hatching success was 76.1% (N=59 clutches) (Fletcher, Upper Thames Conservation Authority, personal communication). While no other data are available on normal hatching rates for eastern spiny softshell turtles, hatch rates are greater than or equal to 90% in snapping turtles at low contamination reference sites.

In 1998, infertile eastern spiny softshell eggs were collected from the Thames River, Long Point and Rondeau Provincial Park in the Canadian Lake Erie watershed. Contaminant concentrations in infertile eggs showed distinct geographic variation among areas. Eggs from Long Point were the most contaminated, followed by eggs from the Thames River and Rondeau. Eggs from Long Point contained total PCB-congener specific concentrations of 0.07 to 3.0 ug/g wet weight (N=13 clutches); Thames River eggs contained 1.21-1.57 ug/g wet weight (N=4 clutches) and Rondeau eggs contained 0.45-1.6 ug/g wet weight (N=5 clutches) (Canadian Wildlife Service, 1999). Concentrations of p,p'-DDE were lower than PCBs but higher than all other organochlorine pesticides measured in eastern spiny softshell eggs. Concentrations of p,p'-DDE at Long Point ranged from 0.038 to 0.577 ug/g, Thames River eggs contained 0.265-0.401 ug/g and Rondeau eggs contained 0.075 to 0.305 ug/g (Canadian Wildlife Service, 1999). However, statistical analysis did not indicate any negative correlation between contaminant levels in eggs and rates of infertility in this species.

In the Great Lakes, Craig Campbell made the earliest collections of reptiles for contaminant analysis in 1974 under contract to the Canadian Wildlife Service (Campbell, 1975; Ontario Research Foundation, 1975). Among the turtles sampled in 1974, the highest contaminant concentrations were in eastern spiny softshell eggs from Rondeau Provincial Park, Lake Erie (0.72 ug/g p,p'-DDE; 5.68 ug/g Aroclor 1254:1260). To date, the concentrations of p,p'-DDE in the eastern spiny softshell eggs from Rondeau Provincial Park in 1974 in Lake Erie are the highest ever found in any reptile egg (Meyers-Shone and Walton, 1994). Maximum mercury concentration among all reptile samples collected in 1974 was 0.39 ug/g (Bishop and Gendron, 1998).

6.3.3 Lake Erie Water Snake

In 1998, plasma was collected from four male Lake Erie water snakes from the shoreline of Pelee Island. The samples were pooled into one sample and total congener-specific PCB concentration in plasma was 0.167 ug/g wet weight. Also in 1998, plasma samples from four male northern water snakes (*Nerodia sipedon*) from Little Lake in north-central Ontario (reference site) contained 0.012 ug/g PCBs and plasma of four female northern water snakes from eastern Lake Ontario contained 0.003 ug/g PCBs. Concentration of p,p'-DDE was highest among concentrations of 21 organochlorine pesticides measured in those plasma samples but p,p'-DDE levels were still low, ranging from 0.002 to 0.005 ng/g wet weight among sites (Canadian Wildlife Service, 1999).

Water snakes are ectothermic (a.k.a. cold-blooded) and therefore contaminant levels would be expected to be lower than in endothermic (a.k.a. warm-blooded) animals such as birds who must consume more food per body weight to maintain relatively higher body temperatures. However, the wet weight concentration of congener-specific PCBs in water snakes on Pelee Island are similar to those reported in plasma of nestling Bald Eagles in Lake Superior (USA), lower Columbia River and inland Wisconsin (Bowerman *et al.* 1990; 1994a; Dykstra *et al.* 1998). These concentrations are comparable on a wet weight basis but water snake plasma contained twice as much lipid as Bald Eagles (Elliott *et al.* 1998). Still, these findings and earlier results in 1974 indicate that the endangered Lake Erie water snake is exposed to considerable PCB levels in its diet from western Lake Erie.

6.3.4 Summary of Impairments and Contaminant Effects – Lake Erie Reptiles

- Few studies exist that examine both the levels and associated effects of contaminants on reptiles living in the Lake Erie watershed. The few studies that exist for Lake Erie have primarily examined contaminant concentrations in tissues and eggs.

- Contaminant concentrations in snapping turtle eggs from Big Creek Marsh (Long Point, Ontario) and Rondeau Provincial Park, Ontario are not associated with elevated rates of embryo toxicity or deformities in this species. Concentrations of PCBs detected in eggs from two sites in Ohio (Ottawa River and Ottawa National Wildlife Refuge) are similar to those from Hamilton Harbour where reproductive anomalies have been correlated with PCB exposure in snapping turtle eggs.
- Feminization effects noted in adult male snapping turtles from Long Point, Ontario may indicate subtle endocrine disrupting effects in this species.
- Contaminant concentrations in Lake Erie water snakes from Pelee Island suggest an examination of potential health effects of contaminant exposure is warranted. The Canadian Wildlife Service, University of Guelph, and World Wildlife Fund initiated such a study in 1999.
- Contaminant levels in the eggs of the threatened eastern spiny softshell turtle and the corresponding low rates of hatching in the Ontario Lake Erie basin suggest further investigation of contaminant effects is warranted. The Canadian Wildlife Service, World Wildlife Fund, and Upper Thames Conservation Authority initiated such a study in 1999.

6.4 Amphibians

6.4.1 Anurans (Frogs and Toads)

Chlorinated Hydrocarbons in Frogs and Toads

There has been little study of the effects of contaminants on amphibians in the Great Lakes basin (Bishop and Gendron 1998).

The intense use of DDT and other organochlorine pesticides to combat mosquitoes in Point Pelee National Park in the 1950s and 1960s has resulted in persistent p,p'-DDE concentrations occurring in amphibians collected in the 1990s. Maximum concentrations were 1.01 ug/g lipid weight p,p'-DDE whole body samples of spring peepers (*Pseudacris crucifer*). There is concern, but no definitive proof that DDT exposure may have led to reproductive impairment or reduced adult survival in many amphibians. It is difficult to be precise, since toxicity of DDT and dieldrin to amphibians is not well understood at present.

In Point Pelee National Park, there has been no marked change in the amount of wetland habitat available over the past 100 years, although species diversity in plants within the marsh has changed. During that period, four species of amphibians were extirpated: bullfrog (*Rana catesbeiana*), gray treefrog (*Hyla versicolor*), northern cricket frog (*Acris crepitans*), and Fowler's toad (*Bufo fowleri*). Those species disappeared during the period of DDT use (Hecnar and McCloskey 1996, Russell *et al.* 1995). Laboratory studies in Great Britain have provided some evidence for deformities at contaminant levels similar to those that were likely present in some Pelee wetlands (Cooke 1970, 1971, 1972, 1973, 1974, 1979, 1981). More research is needed on native North American species to confirm any possible effects.

In the mid-1990s, concentrations of organochlorines were measured in green frogs (*Rana clamitans*) and northern leopard frogs (*Rana pipiens*) from locations in the Canadian side of the Lake Erie watershed (Table11) (Gillan *et al.* 1998). Sediment extracts from the sites were tested for cytotoxicity and genotoxicity on a leopard frog embryo cell line. Genotoxic effects of contaminants were examined using DNA strand break tests on leopard frog embryo cell line. No significant genotoxic stress was found (Gillan *et al.* 1998). Cytotoxicity was tested on a leopard frog embryo cell line using sediment extracts from the sites sampled for green frogs. Cytotoxicity was detected at 200 g sediment equivalents per litre of culture medium from Ojibway Provincial Park and three sites on Lake Ontario (Gillan *et al.* 1998). Field surveys revealed viable populations at all of the sites (Gillan *et al.* 1998).

Table 11. Arithmetic mean contaminant concentrations in whole body samples of green frogs and northern leopard frogs (Gillan *et al.* 1998).

Location	pp'-DDE (ug/kg lipid wt.) Green Frog	pp'-DDE (ug/kg lipid wt.) N. Leopard Frog	Aroclor 1254: 1260 (ug/kg lipid wt.) Green Frog	Aroclor 1254: 1260 (ug/kg lipid wt.) N. Leopard Frog
Long Point, Ontario	252	659	986	306
Rondeau Provincial Park, Ontario	179	179	885	885
Ojibway Provincial Park, Windsor	156	125	950	559
Hillman Marsh, near Leamington, Ontario	284	No collection	1,699	No collection

Nitrogen Pollution Risk Assessment

Nitrogen pollution from anthropogenic sources enters water bodies through runoff associated with nitrate fertilization, livestock, precipitation, and effluents from industrial, human and animal wastes. A review by Rouse *et al.* (1999) evaluated the risk of direct and indirect effects of nitrate on amphibian populations. This review used a simple comparison of known environmental nitrate concentrations in North American waters to nitrate concentrations known to cause toxicity in a laboratory setting to amphibian larvae and other species that play an important role in amphibian ecology.

Lethal and sublethal (a reduced rate of growth and development) effects in amphibians are detected in laboratory tests at nitrate concentrations between 2.5 and 385 mg/L (Table 12).

Furthermore, amphibian food sources (such as insects) and predators (such as fish) are also affected by elevated levels of ammonia and nitrate in surface waters (Rouse *et al.* 1999), which may have important implications for the survival of amphibian populations and food webs in general.

Table 12. The toxicity of nitrate to amphibians (Rouse *et al.* 1999).

Species	Stage	Endpoint	Concentration of Nitrate (mg/l)
Bufo americanus	Tadpole	96h-LC50	13.6 & 39.3
Pseudacris triseriata	Tadpole	96h-LC50	17
Rana pipiens	Tadpole	96h-LC50	22.6
Rana clamitans	Tadpole	96h-LC50	32.4
P. triseriata	Tadpole	developmental	2.5-10
R. pipiens	Tadpole	developmental	2.5-10
R. clamitans	Tadpole	developmental	2.5-10
Bufo bufo	Tadpole	96h-LC50	385
Bufo bufo	Tadpole	developmental	9
Bufo bufo	Tadpole	death	22.6
Litoria caerulea	Tadpole	developmental	9
Litoria caerulea	Tadpole	death	22.6
*Rana temoraria**	Adult	EC50-paper	3.6 g/m2
Rana temoraria	Adult	EC50-soil	6.9 g/m2

* Frogs were placed on moist paper or soil spread with ammonium nitrate granules.

Environmental concentrations of nitrate in surface waters in agricultural watersheds in southwestern Ontario and USA states in the Lake Erie watershed ranged from 1 to 40 mg/L. Of 8,000 water samples from rivers in the watersheds of Lake Erie and St. Clair in the Canadian Great Lakes and in USA states in the Lake Erie watershed, 19.8% had nitrate levels above 3 mg/L. This concentration was known to cause physical and behavioral abnormalities in some amphibian species in the laboratory (Rouse *et al.* 1999). A total of 3.1% samples contained nitrate levels that would be high enough to kill tadpoles of native amphibian species in laboratory tests (Rouse *et al.* 1999). Ultimately, there is a need to reduce the use of fertilizers, control the run-off of human and livestock waste through the use of vegetative buffer zones around watercourses, and establish and enforce water quality guidelines for nitrate for the protection of aquatic life.

6.4.2 Mudpuppy

Polycyclic aromatic hydrocarbons (PAHs) were measured in bile of adult mudpuppies (*Necturus maculosus*) sampled during the winters of 1992 and 1993 at Akwesasne and Batiscan River in the St. Lawrence River, Des Prairies River and Quesnel Bay on the Ottawa River, Ontario system; and during the winters of 1994 and 1995 at Wolfe Island (Lake Ontario), Big Creek Marsh at Long Point in Ontario (Lake Erie) and the southern end of the Detroit River (Lake

Erie). Des Prairies River and Detroit River were the two sites showing significant differences in concentrations of biliary 1-hydroxy pyrene when compared to all other sites. Detroit River samples had, by far, the highest concentrations of biliary 1-hydroxy pyrene (median 798 ng/ml). The levels of this chemical were often not detected or close to detection limit (30 ng/ml) in other locations except for samples from Des Prairies River in the Ottawa River watershed, which had a median of 84 ng/ml (Trudeau *et al.* 1998).

PCBs and organochlorine compounds have also been measured in mudpuppies from these sites. Mudpuppies from Big Creek marsh (Long Point, Ontario) in Lake Erie contained low total summed congener concentrations of PCBs (mean = 0.27 ug/g) and organochlorine pesticide concentrations (all means < 0.70 ug/g) in gonads but showed a digital deformity rate of 16% (N=50). The background rate of deformities in this species appears to be <10% at reference sites in the Ottawa River and St. Lawrence River watersheds. Hence, the rate of 16% at Big Creek (Long Point, Ontario) is considered elevated compared to other sites. Samples from the Detroit River contained mean concentration of 2.28 ug/g total of PCB congeners and < 0.24 ug/g organochlorine pesticides in female gonads and showed deformity rates of 32.9% (N=70; 35 females and 35 males) (Bishop and Gendron, personal communication). These samples have not been analyzed for dioxins and furans, to date. At present, the cause of deformities in mudpuppies is still under investigation. It is suspected that the deformities may have an environmental chemical etiology but there may also be an underlying natural cause in sites such as Long Point.

The sensitivity of mudpuppies, frog tadpoles, and adult frogs to 3-trifluoromethyl-4-nitrophenol (TFM) use in the Great Lakes has been noted on many occasions (Gilderhus and Johnson, 1980). TFM has been used historically in 19 (8 in USA/11 in Canada) of the 842 tributaries to Lake Erie for sea lamprey (*Petromyzon marinus*) control. Since 1995, TFM has been applied once in Conneaut Creek and the Grand River in Ohio and Big Creek and Big Otter Creek in Ontario. Only four Lake Erie tributaries (Big Creek, Ontario and three USA tributaries) are currently scheduled for future regular treatments every four to six years (Rob Young, personal communication).

When TFM is used, amphibians have regularly been found dead in creeks immediately after treatment in Lake Erie watersheds and elsewhere in the Great Lakes (Gilderhus and Johnson, 1980, Matson, 1990). Laboratory tests have confirmed that species native to the Great Lakes basin such as gray tree frog, leopard frog and bullfrog are sensitive to field applied rates of TFM (Chandler and Marking, 1975). In the Grand River, Ohio, Matson (1990) found that mudpuppy population size decreased by a minimum of 29% after a spray event in 1987. In 1999, the Grand River was sprayed with TFM and dead mudpuppies were found downstream of the spray zone within twenty-fours hours after the spray event (Matson, personal communication).

Because TFM is not bioaccumulative and is only applied periodically in closely controlled and monitored conditions, the associated mudpuppy mortality is often perceived to be insignificant. However, mudpuppies do not become sexually mature until four to six years of age. Given the projected schedule for future TFM applications, there is the potential for the TFM applications to match periods when large numbers of mudpuppy are reaching an age when they can reproduce. In addition, TFM is generally applied in the spring when stream flows are higher. Therefore, TFM has the potential to kill a portion of the existing females before they lay their eggs in May and June.

6.4.3 Summary of Effects of Contaminants on Lake Erie Amphibians

- Contaminant concentrations, including organochlorine and polycylic aromatic hydrocarbons, have been measured in amphibians at various locations in the Canadian Lake Erie basin. There is no long-term data set available for any species or site.

- Studies of biological effects on amphibians are sparse. The presence of p,p'-DDE [1.01 ug/g lipid wt. p,p'-DDE] in some amphibians from Point Pelee National Park in Ontario suggest that further study is required to determine the sensitivity of native Lake Erie amphibians to p,p'-DDE. Studies of biological effects of contamination in mudpuppies are ongoing and results are pending. Deformity rates of mudpuppies at Long Point and Detroit River are elevated well above the background rates reported for inland areas of the Great Lakes and St. Lawrence River basin. However, a contaminant-associated etiology has not been confirmed at this time.

- Nitrate concentrations in agricultural watersheds of Lake Erie (3.1% of water samples) are high enough to exceed the LC50 (lethal concentration that kills 50% of test population) or sublethally affect (a reduced rate of growth and development; 19.8% of water samples) amphibian tadpoles of various species. However, these predictions are based on laboratory-based studies and need to be tested in wild populations.

- The continued use of TFM is certain to kill amphibians in the watersheds in Lake Erie, where it is still used. Population effects of TFM on amphibians have been noted in the Grand River, Ohio (the only site studied to date).

7. HAZARD ASSESSMENT OF CURRENT AMBIENT CONCENTRATIONS OF PCBS, TCDD, AND TCDD-EQS IN LAKE ERIE WATER FOR FISH-EATING WILDLIFE.

To supplement the impairment assessments of the previous sections, this section provides a risk assessment to compare ambient water concentrations of particular contaminants to concentrations that have been shown to be 'safe' (i.e., NOECs or No-Observed Effect Concentrations) in laboratory and (or) field studies on a particular species. This intuitive approach to a hazard assessment makes a simple calculation based on species-specific exposure and effects data. For some species (Bald Eagle, Herring Gull, cormorant and mink), there are sufficient field and laboratory data to determine 'safe' concentrations in eggs or diets that do not cause reproductive impairments or deformities. These 'safe' concentrations or NOECs can be divided by field-derived, species-specific bioaccumulation factors (BAFs) to calculate the maximum safe water concentrations. These calculated safe water concentrations can be compared to actual ambient water concentrations to determine the risk presented by these ambient concentrations. Impairment conclusions based on this risk assessment approach are summarized in Table 13 at the end of this section.

The traditional approach to generating scientifically-defensible risk assessments for toxic substances has been to generate toxicity tests on each substance or generic group of substances (e.g., PCB Aroclor mixtures) with surrogate mammalian test species, apply an expected bioconcentration or bioaccumulation factor and develop the plausible human exposures based on ingestion or dermal uptake by the most exposed group of people. Typically, the estimation of effects on human health was based on the endpoint of induction of cancer in the human population at an excess rate of one occurrence in 100,000 persons. For more than three decades, it has been presumed that protecting human health through the use of the human cancer paradigm would automatically protect wildlife. When viewed through the lens of wildlife health there are many problems inherent in this regulatory scheme.

- First, many wildlife species are genetically far more sensitive to toxicants than are the typical rodent surrogate test species (e.g., Hose and Guillette 1995, Tillitt *et al.* 1996). Furthermore, crossing class lines (e.g., generalizing from rodents to egg-laying species) often fails to pick-up on fundamentally different mechanisms of action in wild species compared to test mammal species (e.g., eggshell thinning in birds versus placental mammals).
- Second, the routes and degree of exposures to wildlife are often orders of magnitude greater than in humans. For example, a standard based on humans eating a fish meal once per month where the contaminated biomass is typically less than 1% of the total human diet is a far cry from the piscivorous aquatic bird, otter or mink that consumes virtually 100% of its diet as contaminated foods from an aquatic ecosystem. Also, the human health cancer-based standards are often based on consumption of fat-trimmed fish fillets, whereas wildlife eat the whole fish or invertebrate carcass. These differences can lead to almost three orders of magnitude greater exposures to wildlife depending on a given aquatic system, compared to humans that eat small quantities of the same contaminated resources occasionally and give credence to fish advisories.

- Third, many wildlife species, particularly birds, do not develop neoplasms regardless of their environmental exposures. Wildlife responses to exposures are far more likely to manifest as biomarkers of impairment (e.g., P-450 enzyme induction), reproductive problems, immune suppression and other subtle manifestations rather than cancers in wild species (Gilbertson *et al.* 1991; Fox 1993; Grasman *et al.* 1996).

- Fourth, a huge amount of research documents the much greater sensitivity of embryos and reproductive processes to numerous toxicants compared to the induction of cancer in adult mammals (e.g., Cheung *et al.* 1981; Giesy *et al.* 1994a, b; Ludwig *et al.* 1996). In general, embryos of any given taxon appear to be about a hundred-fold more sensitive to the deleterious effects of environmental toxicants than their parents.

- Fifth, risk assessments based on testing either pure substances (e.g., p,p'-DDT, or on generic mixtures of substances (e.g., total PCBs) do not account for the environmental processes which alter the parent substances or parental mixtures of compounds (e.g., PCB congeners) [See Oliver *et al.* 1989]. Two processes, the production of breakdown products (e.g., DDE from DDT) and the environmental changes in parent mixtures (e.g., selective bioaccumulation of the planar PCBs from parental mixtures) are not accounted for in the testing of single compounds or parental mixtures (Safe 1990; Yamashita *et al.* 1993, Giesy *et al.* 1994a, b). Numerous research papers have confirmed the generalization that mixtures of PCBs and dioxins found in tissues of predators may be from one to six-fold more toxic than the parental mixtures that were originally discharged to the environment (Tillitt *et al.* 1991, 1992; Jones *et al.* 1993 a, b). Further, two bird species eating the same species of Great Lakes fish from the same sites were shown to retain very different congener mixtures causing *in vivo* residues to be three and six-fold more toxic than parent mixtures (Yamashita *et al.* 1993). Selective bioaccumulation can lead to a half-order of magnitude error in projections of toxicity based on parental compounds/mixtures.

- Sixth, modeling of toxic effects in species and populations depends on numerous steady state assumptions that are not likely to be valid in the environment. Of particular interest is the difference between bioconcentration factors (BCFs = the passive uptake of toxic substances from water) and biomagnification factors (BMFs = the metabolic concentration of ingested toxic substances from foods which leads to toxicant retention while the energy in the ingested biomass is metabolized by the predator) [See Clark *et al.* 1988]. Even though few of these BCF/BMF factors are measured accurately for wildlife, there is little reason to believe that human or rodent BCFs/BAFs are accurate predictors of wildlife BMFs because surrogate species are not secondary or tertiary predators as are most vulnerable wildlife. For example, many rodent BAFs are thought to approximate a hundred thousand to a million fold from laboratory experiments, while Great Lakes Caspian Terns and Double-crested Cormorants are measured in the range of three to five million and Bald Eagles about twenty-five million-fold. This is a potential underestimation of the lowest adverse environmental effect levels (LOECs) of approximately two orders of magnitude.

- Finally seventh, many individual compounds have the same mode of action which means that data on compounds or generic mixtures (e.g., Aroclor 1242) individually tested cannot predict environmental toxicity accurately. For example, the planar PCBs, PCDDs, PCDFs, PCNs, PCDTs, and a half-dozen more generic classes of compounds share the dioxin-like mode of action of stimulation of the P-450 enzyme system and numerous other 'dioxin-like' effects (Giesy *et al.* 1994a, b). Other non-planar congeners of these groups have the specific mode of action of inhibiting certain neurotransmitters, especially the dopamine pathway. Others effect changes to the estrogen-androgen axis and related sex hormone pathways, or specific transport proteins, especially the thyroxine and vitamin A transport protein TTR (Brouwer *et al.* 1998; Klasson-Wehler *et al.* 1998). In general, it is believed that compounds sharing the same mode of action are additive in their toxicity when present in the same mixture (Safe 1990). However there are instances where particular congener mixtures are less than additive or even antagonistic for a particular mode of toxic action (Bannister *et al.* 1987). A great deal of progress was made in the last decade to understand additivity/antagonism of mixtures through the development of several bioassay systems for particular modes of action (e.g., H4IIE bioassay for dioxin equivalents of mixtures [TCDD-EQs], standardized tests for immunosuppression in young animals, dopamine bioassays, etc.) [See Giesy and Graney 1989; Tillitt *et al.* 1991 a, b; Giesy *et al.* 1994a, b; Grasman *et al.* 1996; and Kennedy *et al.* 1996 for examples of bioassay techniques.].

In summary, there is a potential for error of four to seven orders of magnitude *underestimation* of the impacts of toxic substances on wildlife when assessments are based on the conventions of human health, single compounds, and the human cancer paradigm. Most jurisdictions still have such traditional approaches in force, although the Great Lakes Initiative standards are changing some approaches. Few state or provincial regulators are fully cognizant of the emerging technologies to remedy these deficiencies. This leads to the basic question of this analysis: "Is there a practical alternative to conventional laboratory and surrogate species-based toxicological approaches to estimating the impacts of toxic substances on wildlife?"

A viable alternative is to learn the suite of biological effects produced by environmental contaminants, measure the low effect levels in wildlife, the bioaccumulation factors of wildlife species and relate the observed body burdens of toxic substances to measured water quality parameters in a given body of water. Effectively, this method avoids modeling and all the assumptions therein and questions of additivity or antagonisms of environmental mixtures. Because it is based on real field responses of real species to real measured concentrations of substances, there is no need to validate observations in the field.

In the upper Great Lakes, Ludwig *et al.* (1993) investigated the real-world responses of six species (five wild species in the aquatic food web and the very sensitive laboratory surrogate White Leghorn Chicken) to measured environmental exposures and related these observations to actual water concentrations of PCBs. They also researched the lowest adverse effect data for each species and estimated the no adverse effect concentrations (NOECs) for each species based on real observed data in the wild environment and a safety factor of 10. For example, among four wild avian species, the LOEC based on total PCBs in eggs and the endpoint of statistically significant reduced egg hatchability ranged from 3.5 to 5.0 mg/kg wet weight. Further, the most sensitive avian species to PCBs is known to be the surrogate species, White Leghorn Chicken; its egg LOAEL was only 0.29 mg/kg. NOAELs for the four wild species were assumed to be an order of magnitude less than observed LOAELs or 0.35 to 0.50 mg/kg in their eggs. Selective bioaccumulation was accounted for, and the actual levels of dissolved PCBs in the waters of the lakes was that observed in wildlife concentrations of PCBs and exceedence values calculated for all of the Great Lakes.

For the four avian species, the ambient 1986 concentration of PCBs in Lake Erie waters produced exceedences in the species above their no effect levels ranging from 45 fold in the Herring Gull to 1,400 fold in the Bald Eagle, assuming all of their food was derived from an open lake food web. In order to produce habitats in the Great Lakes so that all species would be at or below their measured LOAEL, the level of dissolved total PCBs in waters would have to be 1 x 10-15 or one part per quadrillion and the no effect level for all species would be a tenth of that. By comparison, the 1986 dissolved levels in Lake Erie were 0.7 parts per trillion (ng/L) and 1.378 pg/g total in unfiltered water. The exceedence for the five species examined in Lake Erie (assuming all their food came from a Lake Erie food web) were 45 fold for Herring Gulls, 60 fold for Double-crested Cormorants, 81 fold for Caspian Terns, 280 fold for mink and 1,400 fold for Bald Eagles. In essence, to eliminate all adverse biological effects to this group of native aquatic-dependant wildlife species from PCBs, the ambient levels of total PCBs in Lake Erie would have to fall by three orders of magnitude. When recalculated for 1993 data, exceedence values for the western basin were even higher than the 1986 values for all of Lake Erie. Exceedence values in 1993 for the central and eastern basins were lower than values for the western basin, but still indicate significant risks (ambient water concentrations 9-142 times above risk criterion) for all species examined. An implication of the spread in these exceedence values is that PCBs are an important factor controlling the outcome of competition and success amongst Great Lakes native species (Fox 1995; Ludwig *et al.* 1996b).

Table 13. Wildlife risk assessment calculations for total PCBs in Lake Erie.

Species	Lowest Observable Effect Concentration (LOEC)		Endpoint	Reference Dose (mg/kg wet wt.) = LOEC/10[a]	Bioaccumulation Factor (BAF)	Risk Criterion (pg/L) = (Reference Dose/BAF) x 10^{9} [b]	Exceedence Value = PCB concentration in L. Erie water/Risk Criterion		
	(mg/kg wet wt.)	(Tissue or Diet)					1986 L. Erie 1,400 pg/L	1993 western basin 1,550 pg/L	1993 central & eastern basins 285 pg/L
Bald Eagle	4.0	Egg	Egg lethality	0.40	4×10^{8}	1	1,400	1,550	285
Herring Gull	5.0	Egg	Embryonic deformities and egg lethality	0.50	1.6×10^{7}	31	45	50	9
Caspian Tern	4.2	Egg	Embryonic deformities and egg lethality	0.42	2.4×10^{7}	17	81	92	17
Double-crested Cormorant	3.5	Egg	Egg lethality	0.35	1.5×10^{7}	23	60	67	12
White Leghorn Chicken	0.29	Diet	Embryonic deformities	0.029	1.6×10^{7}	2	700	775	142
Ranch mink	0.84	Diet	Kit lethality and reduced growth rate	0.084	1.6×10^{7}	5	280	310	57

[a] 10 is a conservative safety factor

[b] 10^{9} converts from mg/kg to pg/L

8. POTENTIAL RESEARCH ISSUES AND PRIORITY INFORMATION GAPS

Programs and funding for monitoring contaminant concentrations and assessing their biological effects have declined in recent years. Maintenance of these programs is essential for assessing recovery from impairment, detecting the emergence of new problems and filling the information gaps described in this section.

- Most of the major contaminants considered in this report are organochlorines, because research shows that they have caused past and current reproductive impairments and population-level effects. More environmental data are available for this class of chemicals than others. However, other newer industrial chemicals and pesticides are released into the Lake Erie ecosystem in large quantities. Few biomonitoring studies have examined the concentrations and biological effects of these chemicals in Lake Erie wildlife. Recent advances in laboratory and field toxicology have shown that some of these chemicals (e.g., nonylphenol, bisphenol A, atrazine, aldicarb) are able to disrupt the function of the endocrine, immune and nervous systems, often following low level exposure during development.

- Due to improvements in the health of national populations of Bald Eagles in both the USA and Canada, the level of effort to monitor or band Lake Erie Bald Eagles has decreased in recent years. However, contaminant impacts are still affecting the recovery of the Lake Erie sub-population. Therefore, it is important to continue studies of reproductive success, deformities and contaminant concentrations in blood and eggs. It is also important to consider continuing banding/color marking studies to allow tracking of individual eagles from the territories where they are raised to the territories where they breed. Until about two years ago, this was done across the entire lake. Today, due to reduced funding, this type of more intensive monitoring is more spotty and declining. Studies of recruitment patterns will be essential for answering questions about the high turnover rate of adult eagles breeding on the Lake Erie shoreline, the survival and reproductive success of eagles exposed developmentally to contaminants from Lake Erie and the rate of immigration from inland areas to the Lake Erie shoreline.

- The cause of the reproductive impairment in Herring Gulls on West Sister Island requires further investigation. Toxicologically significant concentrations of microcystin toxin have been found in the livers of one Herring Gull from West Sister Island and a number of Caspian Tern chicks from Saginaw Bay, which bears some similarity to western Lake Erie in terms of primary productivity and PCB concentrations. The accumulation of microcystin toxin in colonial waterbirds is an emerging issue that deserves further study. Other potential causes of the reproductive failure include PCB-induced wasting syndrome, infectious disease, or some interaction among these factors.

- A formal deformity survey in Herring Gulls should be initiated to better estimate the rate of deformities. A deformity survey should be conducted in cormorants if significant numbers (i.e., hundreds) nest on the ground in the future.

- Birds, such as Tree Swallows, that eat emergent aquatic insects can accumulate high concentrations of organochlorines and other contaminants. Although studies of Lake Erie Tree Swallows from the eastern and central basins have shown only a few biochemical effects and no reproductive effects, biologically significant impacts are possible in more sensitive species, especially in the western basin where organochlorine concentrations are higher. Such studies should be initiated.

- Little is known about the potential exposure of wild diving ducks to contaminants through consumption of zebra mussels. A significant proportion (52%) of diving ducks (Scaup, Goldeneye, Bufflehead, Scoter and Long-tailed Duck) had zebra mussels in their gizzards at the time of collection (Hamilton and Ankney 1994). A study of the potential for physiological effects following consumption of contaminated zebra mussels is under study through Bird Studies Canada.

- Better information is needed for mink and otter in the following areas: population surveys, tissue residues, and contaminant concentrations in food. The Canadian Wildlife Service is undertaking a mink carcass collection from 1999/2002 within the Canadian Lake Erie watershed. Trapper-caught carcasses from Lake Erie marshes and inland tributaries are being analyzed for contaminants and examined histopathologically and morphologically. Measurements of reproductive organs will be made to determine possible contaminant effects on reproductive development.

- Few studies exist that examine both the levels and associated effects of contaminants on reptiles living in the Lake Erie watershed. The few studies that exist for Lake Erie have primarily examined contaminant concentrations in tissues and eggs. Contaminant concentrations in Lake Erie water snakes from Pelee Island are high enough to justify a study of health and reproductive effects.

- Contaminant concentrations in the threatened eastern spiny softshell and the corresponding low rates of egg hatching in the Lake Erie basin suggest that further investigation of contaminant effects is warranted. The Canadian Wildlife Service, World Wildlife Fund, and Upper Thames River Conservation Authority recently initiated such a study.

- Further investigation of contaminant levels and effects in the common snapping turtle is warranted in coastal wetlands of Lake Erie, especially the western basin and marshes in the USA. Hatching success and deformity rates should be examined. The University of Guelph and the Canadian Wildlife Service are studying other endpoints, such as differential effects on males versus females and behavioral effects in snapping turtles from Lake Ontario and the St. Lawrence River. These endpoints could be examined in Lake Erie populations in the future.

- Data are needed about the sensitivity of amphibian eggs, larvae and adults to DDT concentrations presently occurring in water and the food web of coastal wetlands, especially in Point Pelee National Park.

- There are conflicting opinions about the significance of non-target species sensitivity, particularly mudpuppy, to TFM (when used for sea lamprey eradication) and its implications for potential impairment. Therefore, the impact of TFM on amphibian populations needs to be assessed by monitoring populations of mudpuppies and other amphibians pre- and post-application. From a reproductive standpoint, it is particularly important to determine if TFM has greater impacts on certain age classes and (or) egg-bearing females.

- Based on laboratory studies, nitrate concentrations in agricultural watersheds of Lake Erie are high enough (3.1% of water samples) to exceed the LC_{50} or sublethally affect (a reduced rate of growth and development; 19.8% of water samples) amphibian tadpoles of various species. The results of these laboratory studies need to be verified in wild populations.

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Hazard Assessment of Current Ambient Concentrations of PCBs, TCDD and TCDD-EQs in Lake Erie Water for Fish-eating Wildlife

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