Final Report

ENDOCRINE BIOMARKERS FOR MONITORING CONTAMINANT-ASSOCIATED HEALTH EFFECTS IN FISH-EATING BIRDS OF LAKE ERIE

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Summary

During the last decade, the study of the effects of endocrine disrupting chemicals on wildlife and humans has emerged as an important subdiscipline of environmental toxicology. Numerous studies have shown that many synthetic and natural chemicals can interfere with hormone systems on the biochemical, cellular, and organismal levels. In the Great Lakes, fish-eating birds such as herring gulls have been studied as "sentinel species" for the effects of environmental contaminants for three decades. Previous studies of Great Lakes birds have demonstrated associations between contaminants (primarily PCBs and DDE) and several endocrine-related health effects including eggshell thinning, feminization of male embryos, enlarged thyroid glands and reduced thyroxine concentrations in the blood, and immunosuppression. The objective of this project was to determine whether there are associations between environmental contaminants and altered endocrine function in fish-eating birds of the Great Lakes with special emphasis on reproductive and thyroid systems.

During 1997-2001, 256 pipping herring embryos and 183 prefledgling chicks were collected at 9 Great Lakes sites and a reference site on the Atlantic coast. Contaminant concentrations were measured in fresh eggs, yolk sacs of embryos, and livers and plasma of chicks. While organochlorine concentrations were generally consistent with previous studies, nonylphenol (NP) was found at surprisingly high concentrations in livers at all sites. NP is the breakdown product of alklyphenolpolyethoxylates, which are surfactants used in textile production, pulp and paper processing, oil and gas recovery, detergents, paints, pesticides, animal feeds, and cosmetics. NP is estrogenic and could affect endocrine function in wildlife. This study suggested that NP is among the most prevalent organic contaminants found in young herring gulls. The mean concentration for all colonies was 0.30 mg/kg (wet wt.), ranging from 0.19 mg/kg in Atlantic coast birds to 0.46 mg/kg in birds from Saginaw Bay (Table 1). Concentrations were relatively high in gulls from western Lake. Considering Great Lakes means, NP concentrations were1.8 fold higher than DDE and 0.54 lower than PCBs. Furthermore, NP concentrations were strongly correlated with PCB concentrations. The ubiquitous presence of relatively high concentrations of NP in young gulls from industrialized and nonindustrialized areas of the Great Lakes, as well as the Atlantic reference colony, raises significant concerns about the accumulation and toxicity of NP in fish-eating wildlife. To our knowledge this is the first report of NP bioaccumulation at trophic levels above fish.

In pipping gull embryos, the sex ratio at the marine reference site (Kent Island) was 50% males, but the ratio was 55% males for all Great Lakes sites pooled together. Several sites had approximately 70% males. While these data are not definitive because samples sizes for some sites are too small to estimate sex ratio with low statistical error, overall they suggest a greater frequency of males at hatching in the Great Lakes. Further investigation is required with larger sample sizes.

An important objective of this LEPF project was to investigate the hypothesis that endocrine disrupting chemicals in the Great Lakes are associated with altered gonadal development in young herring gulls. Gonads were examined microscopically at two ages: pipping embryos and 4 week old chicks. Significant intersite differences among were observed in the histological structure of the testes and gonads of herring gull embryos and prefledglings. Gross abnormalities included nodules of primordial germ cells in the testicular cortex and seminiferous

tubules extended through the testicular cortex. Quantitative histological measures that showed included altered Sertoli cell numbers and seminiferous tubule areas in males and oocyte numbers and morphology in females. These types of alterations are characteristic of the types of effects seen in birds exposed to estrogens, DDT, and PCBs. In this study, there were no linear associations between these abnormalities and total PCBs and DDE measured in eggs or nonylphenol measured in eggs. However, effects at low doses of endocrine disruptors often disappear at higher doses, and interactive effects in complex mixtures of contaminants can be difficult to measure. Furthermore, abnormalities at some sites could have been associated with contaminants other than those that were measured. Future multivariate statistical analyses will be used to examine relationships between gonadal abnormalities and contaminants measured in yolk sacs of individual embryos.

Historically organochlorine contaminants have altered thyroid physiology in Great Lakes herring gulls and that these effects have persisted at some sites into the early 1990's. In this study, young herring gulls at contaminated sites exhibited marked reductions in thyroxine concentrations in the thyroid gland at both ages. Hypothyroidism (low plasma thyroxine) and thyroid enlargement were variable but occurred more frequently in chicks at contaminated sites, suggesting that problems may increase with age.

Problem Identification

There is growing concern among scientists, government officials, and the public over the effects of pollutants on the endocrine system. Numerous studies have shown that many synthetic and natural chemicals can interfere with hormone systems on the biochemical, cellular, and organismal levels. However, many important questions remain and require additional research:

- Which synthetic chemicals are most likely to cause these effects in wildlife and humans?
- To what extent do these effects increase when chemicals are found in complex mixtures, rather than alone, in the environment?
- Which wildlife and human populations experience sufficient exposure to cause biologically significant effects such as impaired health or reproduction?

Prior to the initiation of this project, environmental and public health agencies such as the US Environmental Protection Agency, Environment Canada, Health Canada, and the International Joint Commission (IJC) had recently established programs to evaluate toxic effects on hormone systems. During 1995-97, the Workgroup on Ecosystem Health of the IJC is emphasizing the investigation of these effects in the Great Lakes region. Interest in endocrine disrupting chemicals has continued to increase in the scientific and public policy arenas, and it is expected that the results of this project will be of interest to both communities.

Fish and wildlife studies have provided some of the strongest evidence for disruption of endocrine function by xenobiotic chemicals, including feminization of male alligators and turtles by organochlorines in Florida (Guillette *et al.*, 1994), disruption of sexual development and reproduction in fish by pulp mill effluent (Van Der Kraak *et al.*, 1992), and the alteration of reproductive and stress hormones in birds exposed to crude oil or organochlorines (Rattner *et al.*, 1984). During the last 30 years, numerous physiological and population-level impacts of contaminants have been observed in fish-eating birds of the Great Lakes. Endocrine-related effects observed in the herring gull (*Larus argentatus*) include DDE-induced eggshell thinning (Ludwig and Tomoff, 1966), feminization of male embryos (Fox, 1992), prolonged incubation and altered parental behavior (Fox *et al.*, 1978), enlarged thyroid glands and reduced thyroxine concentrations in the blood (Moccia *et al.*, 1986; Fox *et al.*, 1998), and depletion of vitamin A (retinol; Fox, 1993; Fox *et al.*, 1998). Our 1991-92 study suggested that these effects on the thyroid glands and vitamin A continue at highly contaminated sites, including several sites in western Lake Erie (Fox *et al.*, 1998). The alterations in these endocrine variables in Lake Erie birds were among the largest seen at any Great Lakes sites. Our research has shown that contaminants are associated with altered immunological parameters, including thymic atrophy, suppressed T cell function, altered white blood cell counts, and altered blood plasma proteins (Grasman *et al.*, 1996, 2000a,b, Grasman and Fox, 2001). At highly contaminated sites, T-lymphocyte functions are suppressed in herring gull and Caspian tern (*Sterna caspia*) chicks. Immune alterations are significant in this context because the immune system interacts closely with the endocrine system.

Studies of "sentinel species" such as the herring gull provide important biomonitoring data. Biomarkers are biochemical, physiological, or histological changes that measure effects of, or exposure to, toxic chemicals. Ecotoxicologists use biomarkers to clarify cause-effect relationships between pollution and health effects. Biomarkers demonstrate mechanistic links between contaminants and effects on the organismal and population levels, and they may provide early warning signs for such effects. These methods also provide tools for biomonitoring after pollution controls and cleanup are implemented. Biomonitoring data can help guide policies regarding pollution discharge and remediation. Colonial waterbirds of the Great Lakes have been studied as sentinel species for contaminant effects for more than three decades (Grasman *et al.,* 1998).

While some endocrine effects have been found previously in Great Lakes birds, a detailed study of endocrine biomarkers in Lake Erie birds has not been conducted at current levels of contamination. Hence, this project was proposed to the Lake Erie Protection Fund for the following reasons:

- Many contaminants found in Great Lakes wildlife have been shown to disrupt important endocrine functions in laboratory animals.
- Our preliminary endocrine investigations have found significant endocrine effects in Lake Erie gulls (only a few endpoints were examined), and our more extensive immunological studies have shown immunosuppression in birds from the same areas and at other sites with similar contamination.
- The southern and western shores of Lake Erie contain five Areas of Concern (the Raisin, Maumee, Black, Cuyahoga, and Ashtabula Rivers). During 1992, herring gull chicks at Monroe, Michigan, had low post-hatch survival, and several other gull colonies in western Lake Erie have experienced reproductive problems during the 1990's. Several bald eagle chicks with deformities have been found recently at highly contaminated Lake Erie sites.
- Following the introduction of the zebra mussel into Lake Erie in the mid 1980's, significant changes have occurred in the western Lake Erie food web, including a great decrease in phytoplankton. These changes are influencing contaminant cycling in the pelagic food web. During the 1990's, organochlorine concentrations in western Lake Erie gulls remain high (among the highest in the Great Lakes) and are declining only slowly, if at all (Stow, 1995).
- Finally, studies of highly exposed wildlife populations such as Great Lakes gulls should help to clarify the significance of concerns about endocrine disruption in wildlife and humans.

Objectives

The major objectives of this project were:

- To determine whether there are associations between environmental contaminants and altered endocrine function in fish-eating birds of the Great Lakes with special emphasis on reproductive and thyroid systems.

- To evaluate biomarkers that measure the structure and function of the endocrine system and to develop a battery of sensitive assays for assessing the significance of environmental endocrine disruption and monitoring the incidence and severity of such effects in Great Lakes birds.

- To explore interactions between the endocrine and immune systems in wild fish-eating birds exposed to significant concentrations of organochlorine contaminants.

Significant Results

Contaminant Concentrations—Organochlorines and Nonylphenol

During 1997-2001, 256 pipping herring embryos and 183 prefledgling chicks were collected at 9 Great Lakes sites and a reference site on the Atlantic coast (Kent Island in the Bay of Fundy; Fig. 1). Two colonies in northern L. Huron, Chantry and Double Islands, were Great Lakes reference sites with relatively low concentrations of organochlorines. Three Lake Erie colonies (Monroe, on the western shore; West Sister Island, in the middle of the western basin; and Middle Island, on the eastern side of the western basin). Contaminant concentrations were measured in fresh eggs, yolk sacs of embryos, and livers and plasma of chicks. Measured compounds included organochlorines, nonylphenols, hydroxylated PCBs, heavy metals, and polycyclic aromatic hydrocarbons. This report emphasizes PCBs, DDE, and nonylphenol because they were found in the highest concentrations and are thought to be most toxicologically relevant. Not all contaminants were measured in all tissues, and some tissues were pooled before analyses. Organochlorine concentrations are reported in other sections of this report. An important finding regarding nonylphenol is reported in this section.

Alklyphenolpolyethoxylates (APEs) are surfactants that have been used for the last 50 years in a variety of products and processes, including textile production, pulp and paper processing, oil and gas recovery, detergents, paints, pesticides, animal feeds, and cosmetics. APEs enter aquatic ecosystems through sewage treatment plants and industrial point sources. Nonylphenol (NP) and octaphenol (OP), the breakdown products of APEs, are lipophilic and moderately persistent in the environment. Studies of male caged fish in sewage treatment effluents first suggested that APEs and their breakdown products are estrogenic, and subsequent laboratory studies have demonstrated the NP and OP can mimic estrogen in fish, mammalian, and *in vitro* systems. Numerous studies have documented NP concentrations in water and sediment samples, but few studies have reported NP concentrations in fish tissues, but the few reported values for NP in fish tissues suggest the possibility for significant bioaccumulation at higher trophic levels such as fish-eating birds.

Few data available are on NP concentrations in the Great Lakes ecosystem. While NP is often undetectable in Great Lakes water samples, its presence is consistently detected in sediments at concentrations ranging from 0.17-72 mg/kg (dry wt.; Bennie et al., 1997). The only report of NP concentrations in Great Lakes (Lake Michigan) fish tissues (body) was 0.010 mg/kg (wet wt.), although concentrations of fish of the Kalamazoo River, a L. Michigan tributary, were as high as 0.029 mg/kg (Keith et al. 2001). **Because of the presence of APEs in the Great Lakes ecosystem and their potential for bioaccumulation and disruption of endocrine function in fish and wildlife, NP concentrations were measured in pooled liver samples of prefledgling herring gulls from the Great Lakes and an Atlantic Ocean reference site.**

NP was found at surprisingly high concentrations in livers at all sites, suggesting that NP is among the most prevalent organic contaminants found in young herring gulls. The mean concentration for all colonies was 0.30 (sd 0.10) mg/kg (wet wt.), ranging from 0.19 mg/kg in Atlantic coast birds to 0.46 mg/kg in birds from Saginaw Bay (Table 1). **Concentrations were relatively high in gulls from western Lake Erie (0.38 mg/kg at West Sister Island and 0.28 mg/kg at Monroe). Considering Great Lakes means, NP concentrations were1.8 fold higher than DDE and 0.54 lower than PCBs. Furthermore, NP concentrations were strongly correlated with PCB concentrations (r_6=0.99, P<0.001; Fig. 2), suggesting that the uptake and (or) retention of these two chemicals may be linked.** While little is known about toxic effect concentrations in birds, NP has potent estrogenic effects in fish, mammals, and *in vitro* systems. The presence of NP during embryonic development and nestling growth could significantly affect development of the reproductive system, and chronic exposure in adults could impair reproductive function. **The ubiquitous presence of relatively high concentrations of NP in young gulls from industrialized and nonindustrialized areas of the Great Lakes, as well as the Atlantic reference colony, raises significant concerns about the accumulation and toxicity of NP in fish-eating wildlife. To our knowledge this is the first report of NP bioaccumulation at trophic levels above fish.**

Sex Ratios and Identification of Genetic Sex

A recent study of humans who ate L. Michigan fish showed that men with plasma PCB concentrations above 8.1 µg/L fathered more boys (57%) compared to those with lower PCB exposure (45%; Karmaus *et al.,* 2002). The collection of large numbers of embryos and young allowed us to calculate sex ratios at two ages. **In pipping gull embryos, the sex ratio at the marine reference site (Kent Island) was 50% males, but the ratio was 55% males for all Great Lakes sites pooled together. Several sites had approximately 70% males,** including West Sister Island (western Lake Erie), Scotch Bonnet Island (L. Ontario), and Strachan Island (St. Lawrence Seaway). Although sample sizes were small (n=10/site) for Scotch Bonnet and Strachan, the sample size was large (n=39) at West Sister, giving stronger support for the skewed ratio at that site. Other western Lake Erie colonies showed a smaller skew toward males (58% at Middle Island) or no skew (50% at Monroe).

Despite a higher frequency of males at pipping, overall there were fewer males at 4 weeks of age (42% males at the marine reference site and 42% for all Great Lakes sites together). **This suggests differential mortality after hatch with a greater effect on males.** The differential mortality may be greater in the Great Lakes, which start with a higher proportion of males at hatch but have a similar proportion of males at 4 weeks when compared to the marine reference site. Scotch Bonnet Island had only 24% males at 4 weeks. This island noteworthy because high rates of gonadal abnormalities were observed there in the 1970s and the 1990s (this study; see below).

While these data are not definitive because samples sizes for some sites are too small to estimate sex ratio with low statistical error, overall they suggest a greater frequency of males at hatching in the Great Lakes. We are currently collaborating with the Canadian Wildlife Service (D.V. Weseloh) to examine sex ratios at hatching using larger sample sizes. We are using a nonlethal technique that determines the genetic sex of a bird using only a small blood sample. This protocol was developed as part of this LEPF study.

Abnormalities in Gonadal Development

Although there has been relatively little research on birds compared to mammals, it is clear that the development of the reproductive tract in birds is sensitive to hormones and hormone-like substances. In males, such abnormalities include the presence of ovarian tissues in the testes and the development of oviducts, which are usually found only in females. In females, estrogenic or anti-androgenic chemicals may cause the right oviduct to persist. Normally the right oviduct atrophies and only the left oviduct becomes functional. High doses of DDT and estradiol (0.5-20 ppm) injected into Western and California gull eggs caused persistent right oviducts in females and elongated, flattened left testes, or ovotestes within males. Ovotestes contain ovarian cortical tissue with primordial germ cells (PGC) in the abnormal cortex of the gonad (Fry *et al.*, 1987; Fox, 1992). In the Great Lakes during the 1970s, ovotestes were observed in 71% of hatching male herring gull chicks from Scotch Bonnet of L. Ontario. Persistent right oviducts were observed in 56% of hatching females (Fox, 1992). These observations resembled altered reproductive development due to estrogenic and (or) androgenic exposure (Boss and Witschi, 1947). **A major objective of this LEPF project was to investigate the hypothesis that endocrine disrupting chemicals in the Great Lakes are associated with altered gonadal development in young herring gulls.** Gonads were examined microscopically at two ages: pipping embryos and 4 week old chicks. Gonads were examined in two ways: observations of gross abnormalities noted above, and quantitative counts of specific cell types and areas. **These quantitative methods have not been applied frequently in previous studies of gonadal abnormalities in fish and wildlife and represent a new way of assessing the effects of endocrine disruptors on the developing reproductive system.**

<u>Embryos</u>

<u>Gross Abnormalities</u>

No differences were observed in testes and ovary masses from Great Lakes embryos compared to birds from Kent (Tables 3 and 4). **Reproductive tracts (i.e., ducts associated with the gonads) of all embryos were normal. No persistent right oviducts were observed such as when gull eggs were injected with diethylstilbestrol (DES; Boss and Witschi, 1947; Fry *et al.*, 1987).**

In males, primordial germ cells are usually found in the gonadal medulla (central tissue), compared to the cortex (outer layer) in females. Testicular nodules are defined as groups of primordial germ cells found in the testicular cortex, which suggest some degree of feminization of the testis. **Nodules containing ovarian primordial germ cells were generally found at higher frequencies in males from polluted Great Lakes sites** (Monroe 20%, West Sister 27%, Middle 18%, Toronto Harbor 14%, Strachan 14%) than in males from Kent (7%). Male embryos from Chantry Island, where PCB and DDE concentrations were low, had the highest frequency of nodules (33%). NP concentrations were similar at Kent and Chantry. As for sex ratios, small sample sizes of males at some sites reduced the precision of estimates of nodules rates, resulting in low statistical power for intersite comparisons. Nodules have been documented as a transient structure present during development of most bird species including the chicken, robin, sparrow, tern, and duck (Swift, 1916; Riddle and Dunham, 1942; Lahr and Riddle, 1945; Romanoff, 1960). **However, other studies have shown that exposure to estrogenic chemicals or organochlorines can increase the frequency of nodules.** Estrogens are known to cause ovotestes or nodules in developing male chickens (Scheib, 1983). O,p-DDT injected into seabirds collected from clean sites at 2 ppm produced nodules in 88% of males (Fry et al., 1987). Nodules were also documented in common terns (Hart, 1998) and gulls exposed to organochlorines in the wild (Fox, 1992). **Other abnormalities observed in male embryos from polluted sites included seminiferous tubules extending to the outside the capsule of the testis and abnormal medullar tissue outside the testis capsule** (Figs. 4 and 5). Similar abnormalities (i.e., nodules and abnormal seminiferous tubules) were observed in black guillemots exposed to PCBs in Saglek Bay, Labrador (Grasman, unpublished data).

Quantitative Histology

This study used specific histological endpoints to detect structural abnormalities of gonads (Tables 3 and 4). Males from Chantry Island and the North Channel had 5X fewer Sertoli cells compared to males from Kent (Table 3), while Middle Island males had 1.5X more. Reduced Sertoli cells at Chantry and North Channel was unexpected since PCBs were fairly low. Sertoli cells support the development of spermatozoa, and reduced Sertoli number is associated with reduced sperm number and therefore reduced fertility (Sharpe *et al.,* 1995).

Spermatozoa are produced in the seminiferous tubules. Average tubule diameter was decreased in Middle and Toronto Harbor males compared to Kent males (Table 3). Seminiferous tubules develop by forming an opening in the center called a lumen (Fig. 3). Lumen development was greatest in Chantry and Scotch males compared to Kent males. The lumen is needed for spermatogonia to mature into spermatocytes and provides the network for mature sperm to traverse later in development. No significant intersite differences were observed in primordial germ cell number (PGC), capsule area or capsule thickness.

Within females, oocyte number was reduced by 93% in North Channel and 95% in Chantry females compared to Kent females (Table 4). Primordial germ cells (PGC) were marginally reduced in North Channel females compared to Kent females. Oocytes and primordial germ cells are important for normal follicle growth later in development (Fig. 6). Cortex area was significantly reduced in females from Scotch Bonnet, which had high concentrations of mirex and DDE and moderate concentrations of PCBs. The cortex is the area for oocyte and follicle development in the avian ovary. In this study, quantitative histological endpoints revealed ovarian abnormalities not observed by the gross appearance and mass of the ovary.

Prefledgling Chicks

Gross Abnormalities

No differences were observed in testes and ovary masses from Great Lakes chicks compared to gonadal masses from Kent (Tables 5 and 6). Reproductive tracts (i.e., ducts associated with the gonads) of all chicks were normal, except for one male from Scotch Bonnet with an enlarged and flattened ovotestis containing distinct testicular and ovarian tissue (Fig. 8). Persistent right oviducts were not observed in chicks as when gulls were injected with high doses of estrogen and DES (Boss and Witschi, 1947; Fry *et al.*, 1987).

Nodules, or residual ovarian primordial germ cells in the cortex of the testis (Fig. 9), were observed in prefledgling males, although small sample sizes at some sites reduced the precision of estimated frequencies. Nodules were found in 15% of juvenile Kent males (Table 2). Nodules were present in 14% of Monroe, 22% of Saginaw Bay, and 50% of West Sister males. No nodules were found in Chantry or Scotch Bonnet males, although sample sizes were lower for these sites. Examining nodule rates at two reproductive development time points (hatching and prefledgling) suggested that nodules can persist until 4 weeks of age in herring gulls.

Quantitative Histology

Quantitative analysis of histological endpoints revealed that Sertoli cells were decreased 33% in Monroe males compared to Kent (Table 6). Monroe is contaminated with PCBs, PAHs, heavy metals, and NP. Sertoli cells are important in nourishing the primordial germ cells (PGC), which become spermatozoa near puberty. PGCs, which are also important for fertility, were marginally lower in males from Monroe, West Sister, Saginaw Bay, and Chantry compared to Kent males.

Seminiferous tubule diameter was similar among groups compared to Kent males. Seminiferous tubules develop by forming an opening in the center called a lumen. Lumen area was increased 132% in Scotch Bonnet males compared to Kent males, although small sample size prevented an overall significant effect. The increased lumen was also found in Scotch Bonnet embryos. The lumen is needed for spermatozoa to mature into spermatocytes and provides the network for mature sperm to traverse later in development (Fig. 7). **Overall, males from Monroe displayed abnormal testis development due to reduced Sertoli and PGC number. Mirex is higher at Scotch Bonnet, where increased lumen area was observed. The microscopic abnormalities of Monroe and Scotch males indicate that the organization of the testis may have been altered during development with abnormalities persisting to 4 weeks.**

Oocyte number was decreased 21% in Scotch Bonnet and 22% in Monroe females compared to Chantry but not to Kent females (Table 7). Females from Middle had 24% more oocytes than Kent females. Middle females also had 21% more primordial germ cells (PGCs) than Kent females. Cortex or follicle area is where the oocytes develop into follicles later in life and is another important endpoint in reproductive capability (Fig. 10). Follicle area was similar among sites, and therefore, cannot account for a reduced oocyte number in Scotch Bonnet and Monroe females or an increased number in Middle females. These data suggested that the organization of the cortex and the number of oocytes in the ovary were altered during embryonic development that persisted to 4 weeks of age.

Decreased numbers of oocytes undergoing any form of apoptosis may indicate a shift in overall oocyte development. Therefore, oocytes were staged for apoptosis based on structural changes (Table 7). There were no differences in early or late stages of oocyte apoptosis. However, marginal affects were observed in oocytes undergoing late apoptosis by a 23% reduction in oocytes in females from Saginaw Bay and Chantry.

Deposits, lipids or possibly Balbiani bodies, within oocytes may be an indicator of apoptosis (Fig. 11). Deposits were increased 313% in West Sister, 358% in Chantry, and 284% in Middle females compared to Kent females ($p<0.0001$). The Balbiani body is visible only during stages 2 and 3 of a 5-stage development process in primary oocytes of turkeys. Stage 5 of primary oocyte development is characterized by the absence of the Balbiani body with replacement of a layer of lipid droplets (Carlson *et al.,* 1996). Increased oocytes with Balbiani bodies may indicate a stalled stage of development at stage 2 and 3, especially in Chantry and Middle females, where early and late stages of apoptosis were reduced compared to Kent females.

Summary of Alterations in Gonadal Development

In summary, chemical analysis of tissues from Great Lakes sites indicated significant exposure to organochlorines, DDE and mirex. Because tissue concentrations of chemicals are often co-correlated, it is difficult to separate the potential effects of specific chemicals. Significant intersite differences among were observed in the histological structure of the testes and gonads of herring gull embryos and prefledglings. Gross abnormalities included nodules of primordial germ cells in the testicular cortex and seminiferous tubules extended through the testicular cortex. Quantitative histological measures that showed included altered Sertoli cell numbers and seminiferous tubule areas in males and oocyte numbers and morphology in females. These differences suggest developmental reorganization of cells and structures that are important for reproductive function in adulthood. These types of alterations are characteristic of the types of effects seen in birds exposed to estrogens, DDT, and PCBs. In this study, there were no linear associations between these abnormalities and total PCBs and DDE measured in eggs or nonylphenol measured in eggs. However, effects at low doses of endocrine disruptors often disappear at higher doses, and interactive effects in complex mixtures of contaminants can be difficult to measure. Furthermore, abnormalities at some sites could have been associated with contaminants other than those that were measured. Abnormalities were frequently observed at Chantry Island in northeastern Lake Huron, which had relatively low concentrations of PCBs and DDE. Because Chantry Island is close to agricultural areas, it is possible that birds at this colony were exposed to pesticides that were not measured but could have contributed to the gonadal abnormalities. Future multivariate statistical analyses will be used to examine relationships between gonadal abnormalities and contaminants measured in yolk sacs of individual embryos.

Vitellogenin

Vitellogenin is the major egg-yolk protein that is normally produced only by reproductively active adult females in egg laying species (fish, birds, etc.) under the control of endogenous estrogen. However, exogenous estrogenic chemicals can induce vitellogenin production in males and pre-adult females (Robinson *et al.*, 1984). For this project, vitellogenin analysis was conducted by the Canadian Wildlife Service (Dr. Laird Shutt and Mr. Kim Williams). No vitellogenin was detected in the blood plasma of prefledgling herring gulls. These results suggest that these young herring gulls were not exposed to estrogenic chemicals at concentrations sufficient to induce vitellogenin production at this age. It is possible that contaminant-induced vitellogenin production might be induced in other species or in herring gulls at other ages.

Thyroid Physiology

Historically organochlorine contaminants have altered thyroid physiology in Great Lakes herring gulls (Moccia *et al.*, 1986), and that these effects have persisted at some sites into the early 1990's (Fox *et al.*, 1998). Adult herring gulls at contaminated Great Lakes sites have had enlarged thyroid glands (goiter). Histologically these enlarged thyroids exhibited small follicles and increased cell proliferation (microfollicular hyperplasia). In laboratory rodents, PCBs increase the rate of excretion of thyroxine (T_4) from the body (by displacing thyroxine from its transport protein in the blood and by inducing liver enzymes that facilitate excretion). To compensate, the hypothalmo-pituitary system stimulates the thyroid to produce more thyroxine, hence the hyperplasia and enlargement of the thyroid. However, if the rate of excretion remains high, the thyroid gland cannot store up enough thyroxine and the storage follicles shrink. In the most severe cases, the thyroid cannot maintain adequate concentrations of thyroxine in the blood, a condition called organismal hypothyroidism. In the early 1990s, thyroids of adult herring gulls from western L. Erie (Middle Sister and Middle Islands) were approximately twice as large as those in birds from the marine reference site (Kent Island; Fox *et al.*, 1998). Hence, investigation of thyroid physiology was an important part of this LEPF project. We collaborated with Dr. Anne McNabb at Virginia Polytechnic Institute and State University. Dr. McNabb is an internationally recognized expert in avian thyroid physiology. She examined a wide range of variables involved in thyroid function, but here we present only the endpoints that showed significant effects: thyroid weight and thyroxine concentrations in the thyroid gland and blood plasma.

Young herring gulls at contaminated sites exhibited marked reductions in thyroxine concentrations in the thyroid gland at both ages. Effects on the thyroid gland weight and plasma thyroxine concentration varied between ages and depended on year. In pipping embryos, thyroxine concentrations were reduced at all Great Lakes sites, including the 3 western L. Erie colonies (West Sister Island=WS, Monroe=DE, and Middle Island=MI), compared to the marine reference site (Fig. 12). Great Lakes embryos exposed to PCBs and other organochlorines were unable to store up normal amounts of thyroxine in their thyroid glands. During 2000, when PCB concentrations were higher in yolk sacs compared to previous years, the Treat Lake embryos were unable to maintain normal concentrations of circulating thyroxine (Fig. 13), showing organismal hypothyroidism. At this stage of development, enlarged thyroid glands were found only at Strachan Island in 1998 and Toronto Harbor and Monroe in 2000 (Fig. 14).

Prefledgling chicks at all Great Lakes sites exhibited reduced thyroxine concentrations in the thyroid glands in all years (Fig. 15). In some years, chicks at two western Lake Erie sites (West Sister and Monroe) were unable to maintain normal concentrations of plasma thyroxine (Fig. 16), and thyroid enlargement was found at these sites and Saginaw Bay (Fig. 17).

Overall these data indicate that PCB exposure was associated with altered thyroid function in Great Lakes herring gulls. Both embryos and chicks were unable to store normal amounts of thyroxine in the thyroid gland. Hypothyroidism (low plasma thyroxine) and

thyroid enlargement were variable but occurred more frequently in chicks, suggesting that problems may increase with age. Birds exhibiting normal plasma concentrations at the time of sampling might still experience hypothyroidism (low plasma thyroxine) at other times if the thyroid gland is not able to release enough thyroxine from its storage follicles. Hence, hypothyroidism could develop at critical times of development (e.g., hatching and growth, migration).

Reproductive Failures of Herring Gull Colonies

While the focus of this LEPF project was on the endocrine effects of contaminants in Great Lakes gulls, it also provided the opportunity to study reproductive success measured as hatching rates and survival of chicks until fledging (i.e., flight). In some years, reproductive success was very low at several L. Erie (Monroe, West and Middle Sister Islands, Middle Island) and L. Huron (Saginaw Bay) colonies. During the course of this project, low reproductive success was observed in multiple years at all sites except Middle Sister Island, which was only studied one year. The causes of reproductive failures in gull colonies can be diverse: predation, disease, weather, food shortage, and toxic chemicals (human-made pollutants and algal toxins). The specific causes at these sites are unclear, except at Middle Island where predation was important. All of these sites were highly contaminated with PCBs. The failure of the Monroe colony has occurred at or just after hatch, a critical time for the activity of thyroxine-dependent processes. Dr. McNabb has hypothesized that reduced thyroxine production in birds from this site could have contributed to the mortality. **While reproductive success on a regional basis appears to be high enough to maintain herring gull populations throughout the Great Lakes, frequent reproductive failures in western L. Erie and Saginaw Bay suggest problems that should be monitored in the future.**

External Collaboration and Student Support

This Lake Erie Protection Fund Project formed the basis a new set of investigations in our laboratory, all related to toxic effects on the endocrine system. The LEPF project facilitated external collaborations, leveraged funding from other agencies, and provided support for graduate and undergraduate research. Researchers at Wright State University collaborated with scientists from the Canadian Wildlife Service (G. Fox, D. Jeffrey, L. Shutt, K. Williams), US Fish and Wildlife Service (D. Best, L. Williams), Virginia Polytechnic Institute and State University (A. McNabb), and the National Water Research Institute of Environment Canada (D. Bennie). Mr. Fox was obtained funding from CWS's National Wildlife Research Centre and Canada's Toxic Substances Research Initiative to assist with field collections at some Canadian sites and to analyze tissues for contaminant concentrations (including organochlorines, hydroxylated PCBs, nonylphenols, heavy metals, and polycyclic aromatic hydrocarbons) and vitellogenin. (The TSRI grant was $103,500 Canadian). Dr. Anne McNabb received a grant from the USEPA ($256,587) to examine thyroid-related variables in the herring gulls (and in chicken studies, see below).

This LEPF project has directly supported the dissertation/thesis research of one Ph.D. student (M. Elissa Kelly Reaves) in Dr. Grasman's laboratory and two M.S. students in Dr. McNabb's laboratory. Furthermore, the LEPF support has indirectly supported the research of 4 graduate and 4 undergraduate students who have examined other health effects (i.e., other than endocrine) in the herring gulls collected in the LEPF study.

Furthermore, Dr. Grasman and E. Reaves have conducted several spin-off studies related to the LEPF project. The developmental effects of planar PCBs (#s 77 and 126), a PCB mixture (Aroclor 1254), estradiol, and tamoxifen (an estrogen antagonist) have been studied in chicken embryos and young chickens. Gonadal abnormalities have been found in these controlled laboratory studies and should help elucidate and reinforce the results of the herring gull studies. Furthermore, Dr. Grasman and E. Reaves collaborated with on a CWS project on the health effects of PCBs in black guillemots (fish-eating birds) in Saglek, Labrador. The PCBs had been spilled into an otherwise pristine marine ecosystem during renovations at a military radar base. This study provided the unique opportunity to study the toxic effects of PCBs in the absence of other organochlorines, which are co-correlated with PCBs in Great Lakes wildlife. The PCB-exposed guillemots showed gonadal abnormalities that were similar to those found in Great Lakes gulls. Hence, the guillemot study also reinforced the LEPF study.

Information Dissemination

The results of this project will be disseminated to the scientific and Great Lakes pollution policy communities. At least five peer-reviewed publications in the scientific literature will report the results of this project. These manuscripts are in various stages of preparation, but most will be submitted by the end of 2002. Potential journals include Environmental Toxicology and Chemistry, Archives of Environmental Contamination and Toxicology, Marine Pollution Bulletin, and the Journal of Great Lakes Research.

To date, eleven technical presentations about this project have been made at national/international conferences and academic institutions:

Kelly, M.E., K.A. Grasman, and G.A. Fox. 2001. Gonadal histology of Great Lakes herring gull embryos (1997-2000). 2001 meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 11-15, 2001.

Kelly, M.E., K.A. Grasman, and G.A. Fox. 2001. Persistence of gonadal abnormalities in young herring gulls from the Great Lakes (1997-2000). 2001 meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 11-15, 2001.

McNabb, F.M.A., L.A. Fowler, C.M. Parsons, K.A. Grasman, and G.A. Fox. 2001. Herring gulls from PCB-contaminated Great Lakes sites have altered thyroid function. 2001 meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD, November 11-15, 2001.

McNabb, F.M.A., R.J.R. McClearly, L.A. Fowler, C.M. Parsons, K.A. Grasman, and G.A. Fox. 2001. Thyroid function in polychlorinated biphenyl (PCB)-exposed avian embryos and chicks: Field-caught herring gulls and laboratory chickens. 14^{th} International Congress of Comparative Endocrinology, Sorrento, Italy, 26-10 May.

McNabb, F.M.A., L.A. Fowler, C.M. Parsons, K.A. Grasman, and G.A. Fox. 2001. Thyroid function in herring gulls from PCB-contaminated Great Lakes sites. 2001 meeting of the Society for Integrative and Comparative Biology, Chicago, IL, January 3-7, 2001.

Grasman, K.A. 2000. Effects of persistent organic contaminants on the immune and endocrine systems of birds. Invited presentation for the Department of Environmental Toxicology, University of California, Santa Cruz, CA, November.

Kelly, M.E., K.A. Grasman, and G.A. Fox. 2000. Gonadal histology of Great Lakes pipping embryos and 28-day herring gull chicks. 2000 meeting of the Society of Environmental Toxicology and Chemistry, Nashville, TN, November 13-16, 2000.

Kelly, M.E., K.A. Grasman, and G.A. Fox. 2000. Gonadal histology of Great Lakes herring gulls (*Larus argentatus*). 2000 meeting of the Wildlife Society, Nashville, TN, September 12-16, 2000.

McCleary R., F. M.A McNabb, and K.A. Grasman. 2000. Effects of pollutant chemicals on thyroid hormone excretion in birds. 2000 meeting of the Virginia Academy of Science, Radford, Virginia.

Grasman, K.A. 2000. Effects of persistent organic contaminants on the immune and endocrine systems of birds. Department of Biological Sciences, Wright State University, Dayton OH.

Kelly, M.E., G.A. Fox, and K.A. Grasman. 1999. Gonadal histology of Great Lakes herring gulls. 1999 meeting of the Society of Environmental Toxicology and Chemistry, Philadelphia, Pennsylvania.

Seven additional presentations on chicken and guillemot studies related to the LEPF project, as noted above. At least four more technical presentations are anticipated in the future.

These data will be shared with policy and environmental groups such as the International Joint Commission, and Remedial Action Plan (RAP) and Lakewide Mananagement Plan (LaMP) committees. Dr. Grasman has worked with the IJC's Workgroup on Ecosystem Health and the Lake Erie LaMP, including serving as lead author for the following report:

Grasman, K.A., C.A. Bishop, W.W. Bowerman, J.P. Ludwig, and P.A. Martin. 2000. Technical Report 7, Animal Deformities or Reproduction Problems, Beneficial Use Impairment Assessment for the Lake Erie Lakewide Management Plan. (This report is in press as a Technical Report of the Canadian Wildlife Service.)

Dr. Grasman will disseminate the data and conclusions of this LEPF project during his future interactions with the IJC, LaMPs, and natural resource agencies.

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Table 1. Concentrations of nonylphenol, DDE and PCBs in pooled liver samples from four week old herring gull chicks from the Great Lakes and the Atlantic Ocean, 1999 and 2000.

		Concentration (mg/kg wet wt.)		
Site	Year	pp´-DDE	PCB	NP
Kent Is., Atlantic O.	1999	0.015	0.023	0.192
Chantry Is., L. Huron	2000	0.023	0.052	0.205
Saginaw Bay, L. Huron	1999	0.445	1.267	0.474
duplicate	1999			0.454
	Mean			*0.464*
Monroe, L. Erie	1999	0.090	0.463	0.283
West Sister Is., L. Erie	1999	0.082	0.514	0.390
	2000	0.133	1.185	0.371
	Mean	*0.108*	*0.850*	*0.380*
Scotch Bonnet Is., L. Ontario	1999	0.239	0.378	0.233
Great Lakes Mean		**0.181**	**0.602**	**0.323**

Table 2. Site information for herring gull embryos from the Great Lakes and Kent Atlantic reference site from 1997-2000.

Site	Code	N	Location	PCB µg/g	DDE µg/g	Mirex µg/g	Mean Body Mass Male	Mean Body Mass Female
Kent	K	28	Atlantic Coast	1.5	___	___	56.7±2.8	57.8±1.1
N. Channel	NCD	8	Northern L. Huron	0.003	2.7	0.093	48.4±8.5	50.1±2.8
Chantry	C	13	Northern L. Huron	4.6	1.8	0.063	56.8±2.7	56.5±1.2
Scotch	SBI	10	L. Ontario	9.0	6.6	0.91	57.0±2.3	58.9±2.4
Toronto Harbor	TH	14	L. Ontario	9.0	2.7	0.24	54.4±1.3	60.0±1.9
Strachan	STR	10	St. Lawrence	9.4	2.4	0.50	63.1±1.9	59.3±1.7
Monroe, MI	MON	50	Monroe, MI	9.8	1.5	0.041	58.6±1.3	58.3±1.0
West Sister	WS	39	Western L. Erie	13.5	1.7	0.034	55.8±1.0	51.6±2.4
Middle	M	19	Western L. Erie	13.5	1.7	0.034	56.6±2.1	61.0±1.1
Saginaw Bay	SB	38	L. Huron	23.3	6.9	0.074	54.9±1.3	53.9±2.6

Data presented as mean ± standard error of the mean.

Table 3. Means for reproductive endpoints in male herring gull embryos from the Great Lakes and Kent Atlantic reference site from 1997-2000.

Site	N	L. Testis Index	Sertoli # #	Tubule Diameter square/grid	Lumen area square/grid	% males with nodules %
Kent	14	0.005±0.00	0.74 ± 0.12^{AB}	0.96 ± 0.04^{A}	0.07 ± 0.05^{A}	7% (1/14)
North Channel	3	0.005±0.00	0.09 ± 0.04^{A}	0.97 ± 0.13^{AB}	0.15 ± 0.11^{AB}	0% (0/3)
Chantry	9	0.005±0.00	0.15 ± 0.10^{A}	0.99 ± 0.03^{AB}	0.44 ± 0.07^{B}	33% (3/9)
Scotch	7	0.004±0.00	0.53 ± 0.08^{AB}	1.00 ± 0.02^{AB}	0.48 ± 0.06^{B}	0% (0/7)
Toronto Harbor	7	0.004±0.00	0.39 ± 0.13^{AB}	0.75 ± 0.04^{B}	0.00 ± 0.00^{B}	14% (1/7)
Strachan	7	0.003±0.00	0.67 ± 0.22^{AB}	0.93 ± 0.06^{AB}	0.13 ± 0.04^{AB}	14% (1/7)
Monroe, MI	25	0.004±0.00	0.57 ± 0.11^{AB}	0.92 ± 0.02^{AB}	0.11 ± 0.03^{AB}	20% (5/25)
West Sister	26	0.008±0.00	0.53 ± 0.12^{AB}	0.96 ± 0.02^{AB}	0.26 ± 0.04^{AB}	27% (7/26)
Middle	11	0.004±0.00	1.2 ± 0.36^{B}	0.85 ± 0.03^{AB}	0.00 ± 0.00^{B}	18% (2/11)
Saginaw Bay	16	0.004±0.00	0.52 ± 0.14^{AB}	0.91 ± 0.04^{AB}	0.14 ± 0.04^{AB}	6% (1/16)

Data presented as mean ± standard error of the mean. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

Table 4. Means for reproductive endpoints in female herring gull embryos from the Great Lakes and Kent Atlantic reference site from 1997-2000.							
Site	**N**	**Ovary Index**	**% Cortex** %	**% Stroma** %	**Oocyte#** #/grid	**PGC#** #/grid	**Cortex Length** squarea/grid
Kent	14	0.012±0.001	49.0±0.04	51.0±0.04	0.73±0.12	6.9±1.3	24.1±1.9^{A}
North Channel	5	0.010±0.003	33.0±0.10	67.0±0.10	0.06±0.03	3.0±0.8	14.2±4.0^{AB}
Chantry	4	0.017±0.005	24.2±0.03	6.7±0.03	0.04±0.03	3.6±1.0	18.0±5.0^{AB}
Scotch	3	0.011±0.003	26.9±0.01	73.1±0.01	0.38±0.13	5.7±1.2	12.3±3.8^{B}
Toronto Harbor	7	0.011±0.001	55.4±0.08	44.6±0.08	0.23±0.09	5.5±1.9	21.7±3.4^{AB}
Strachan	3	0.008±0.001	46.9±0.07	53.1±0.07	0.78±0.24	5.6±1.5	27.3±5.0^{AB}
Monroe, MI	25	0.011±0.001	43.8±0.03	56.2±0.03	0.63±0.11	5.5±0.7	20.7±1.6^{AB}
West Sister	13	0.010±0.000	50.0±0.03	49.86±0.03	0.35±0.07	6.2±1.2	26.1±2.4^{AB}
Middle	8	0.013±0.001	45.5±0.04	54.5±0.04	0.47±0.14	11.9±2.3	26.1±3.4^{AB}
Saginaw Bay	22	0.011±0.001	52.0±0.04	48.1±0.04	0.85±0.54	6.8±1.2	23.2±1.5^{AB}
Data presented as mean ± standard error of the mean Means with different superscripts are significantly different within the column (p≤0.05).							

Table 5. Site information for juvenile herring gulls from the Great Lakes and Kent Alantic reference site from 1997-2000.

Site	Code	N	Location	PCB µg/g	DDE µg/g	Mirex µg/g	Mean Body Mass Male	Mean Body Mass Female
Kent	K	34	Atlantic Coast	1.5	----	----	732.6±16.9	745.3±18.4
Chantry	C	9	Northern L. Huron	4.6	1.8	0.063	961.8±22.6	727.4±27.3
Scotch	S	16	L. Ontario	9.0	6.6	0.91	937.2±40.5	771.9±25.6
West Sister	WS	30	Western L. Erie	13.5	1.7	0.034	813.7±39.3	733.0±22.2
Monroe, MI	MON	36	Monroe, MI	9.8	1.5	0.041	939.8±30.8	763.4±31.9
Middle	M	2	Western L. Erie	13.5	1.7	0.034	------	775.0±25.0
Saginaw Bay	SB	38	L. Huron	23.3	6.9	0.074	888.6±21.0	755.5±14.0

Data presented as mean ± standard error of the mean.

Table 6. Means for reproductive endpoints in male herring gull chicks (3-4 wk) from the Great Lakes and Kent Atlantic reference site from 1997-2000.

Parameter	Unit	Kent	C	S	WS	MON	SB
L. Testis Index	(testis/BW*100)	0.013±0.001	0.008±0.001	0.012±0.002	0.011±0.001	0.017±0.006	0.010±0.001
Seminiferous Tubule	%	36.3±1.0	29.7±2.4	35.1±3.5	31.5±1.7	32.3±1.7	31.5±1.09
Tubule Diameter	square/grid	1.2±0.05	1.2±.0.06	1.2±0.03	1.1±0.02	1.1±0.03	1.2±0.02
Lumen Area	#/grid	0.14±0.05	0.20±0.08	0.33±0.13	0.19±0.05	0.19±0.04	0.11±0.03
Sertoli cell #	#/grid	2.07±0.18^{AB}	3.06±0.71^{A}	2.37±1.03^{AB}	2.61±0.33^{AB}	1.41±0.18^{B}	2.72±0.47^{AB}
Primordial Germ Cell	#/grid	8.48±0.76	8.55±0.39	9.08±0.81	8.31±0.39	8.64±0.43	9.21±0.45
% individuals with Nodules		15	0	0	50	14	22
Average nodule area		0.42	0	0	0.64	0.63	1.9

Data presented as mean ± standard error of the mean. Sample sizes are: Kent-13, C-5, S-4, WS-12, DE-21, M-0, and SB-18. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 7. Means for reproductive endpoints in female herring gull chicks (3-4 wk) from the Great Lakes and Kent Atlantic reference site from 1997-2000.

Parameter	Unit	Kent	C	S	WS	MON	M	SB
Ovary Index	(ovary/BW*100)	0.045±0.006	0.063±0.009	0.047±0.006	0.056±0.004	0.050±0.005	0.059±0.001	0.058±0.005
Follicle area	%	40.5±1.5	36.4±4.2	31.1±2.6	40.6±0.02	43.3±0.03	29.2±2.8	37.8±0.03
PGC #/follicle area	#	7.18 ± 0.26^{A}	6.31 ± 0.44^{AB}	7.55 ± 0.48^{AB}	6.20 ± 0.26^{AB}	6.78 ± 0.29^{AB}	8.66 ± 0.60^{B}	6.71 ± 0.31^{AB}
Oocyte #/follicle area	#	1.44 ± 0.03^{AB}	1.72 ± 0.05^{A}	1.38 ± 0.08^{B}	1.69 ± 0.07^{AB}	1.34 ± 0.09^{B}	1.89 ± 0.21^{AB}	1.54 ± 0.07^{AB}
Oocytes w/ deposits	#	1.1±0.19	5.04±1.1	1.64±0.51	4.55±0.53	1.49±0.48	4.23±0.47	2.08±0.45
Early apoptosis	%	48.4±4.9	60.3±1.8	39.5±5.9	49.7±4.0	46.8±4.1	52.0±6.4	54.7±2.7
Late apoptosis	%	47.8±5.5	35.3±3.1	53.3±6.4	48.2±4.3	45.7±5.3	42.0±2.4	36.7±2.8

Data presented as mean ± standard error of the mean. Sample sizes are: Kent-21, C-5, S-12, WS-18, DE-15, M-2, and SB-20. Means with different superscripts are significantly different within the row ($p \leq 0.05$)

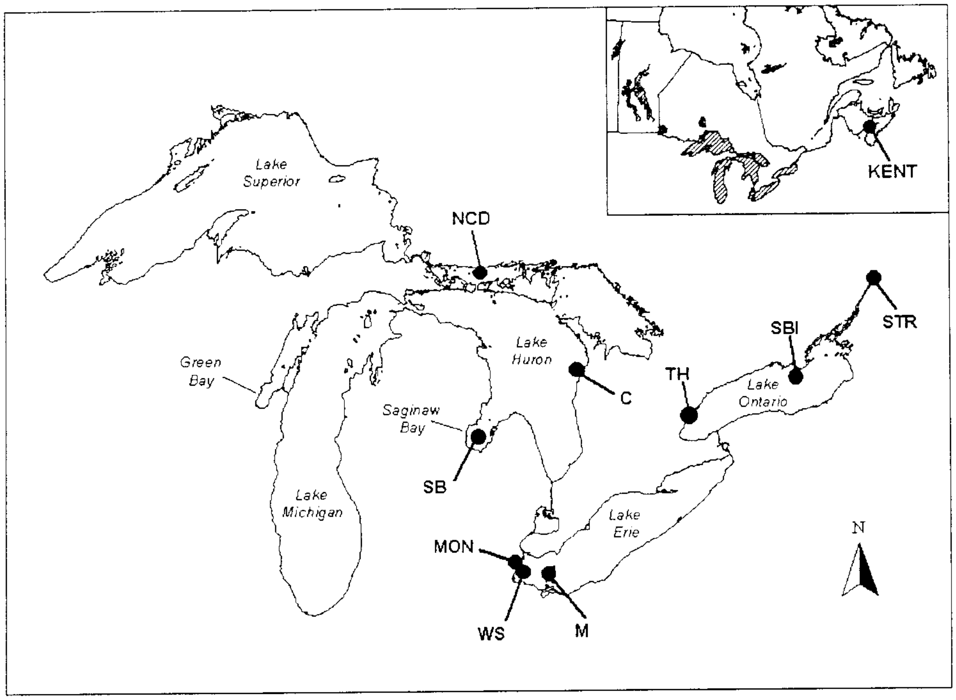

Fig. 1. Locations of herring gull colonies in the Great Lake and on the Atlantic coast where embryos and prefledglings were collected during 1997-2001.

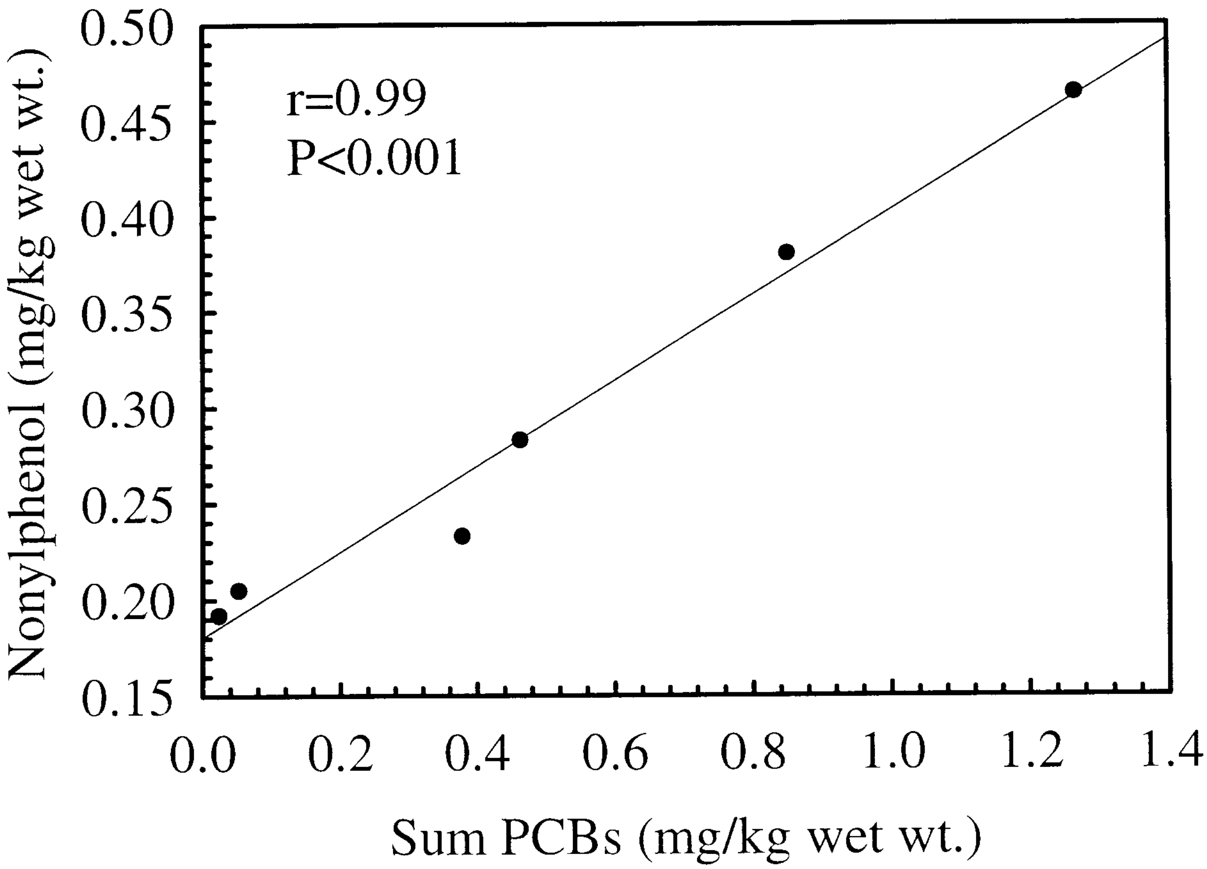

Fig. 2. Relationship between concentrations of nonylphenol and PCBs in pooled liver samples from four week old herring gull chicks from the Great Lakes and Atlantic Ocean, 1999 and 2000.

Fig. 3. Normal medulla and cortex of a testis from a male herring gull embryo from Kent Island, New Brunswick. (toluidine stain, scale bar=100 μm)

Fig. 4. Abnormal seminiferous tubule growth outside the testicular cortex of a male herring gull embryo from Toronto Harbour, Lake Ontario. (toluidine stain, scale bar=100 μm)

Fig. 5. Abnormal testis with seminiferous tubule extending through the testicular capsule of a male herring gull embryo from West Sister Island, Lake Erie (toluidine stain, scale bar=100 μm)

Fig. 6. Normal cortical tissue in the ovary of a female herring gull embryo from Kent Island, New Brunswick. (toluidine stain, scale bar=100 μm)

Fig. 7. Normal medulla and cortex of a testis from a 4 week old male herring gull chick from Kent Island, New Brunswick. (toluidine stain, scale bar=100 μm)

Fig. 8. Ovotestis of a 4 week old herring gull chick from Scotch Bonnet Island, Lake Ontario. (toluidine stain, scale bar=100 μm)

Fig. 9. Nodule in the testicular cortex of a 4 week old male herring gull chick from Saginaw Bay, Lake Huron. (toluidine stain, scale bar=100 μm)

Fig. 10. Normal cortical in the ovary of a 4 week old female herring gull chick from Kent Island, New Brunswick. (toluidine stain, scale bar=100 μm)

Fig. 11. Deposits within oocytes of a 4 week old female herring gull chick from Monroe, MI, on the western shore of Lake Erie. (toluidine stain, scale bar=100 μm)

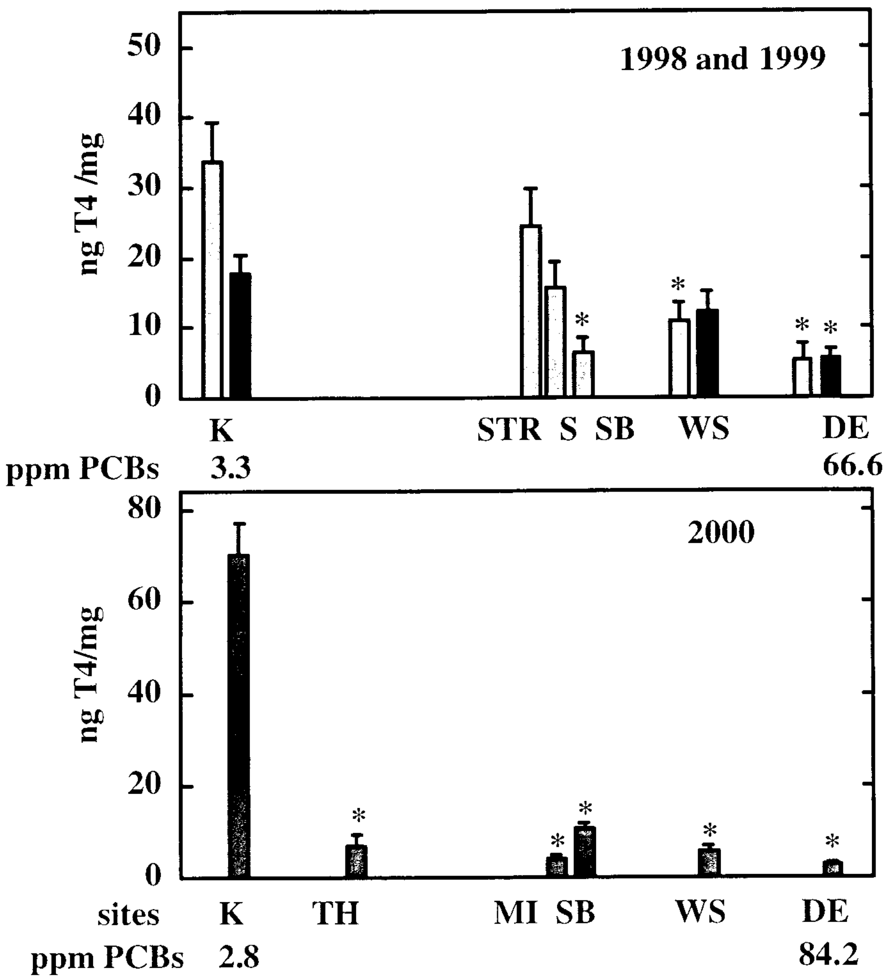

Fig. 12. Thyroid gland T4 content in pipping herring gull embryos from sites in the Great Lakes. Sites are arranged from low to high mean concentrations of PCBs measured yolk sacs of embryos.

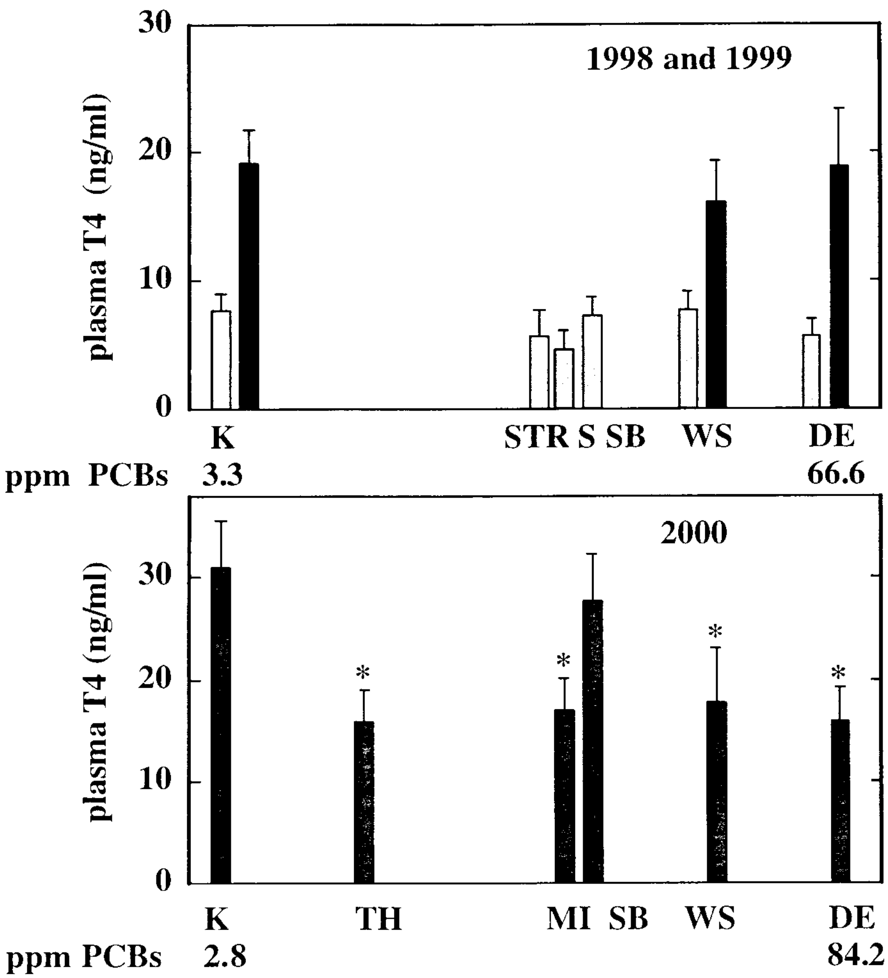

Fig. 13. Plasma T4 in pipping herring gull embryos from sites in the Great Lakes. Sites are arranged from low to high mean concentrations of PCBs measured yolk sacs of embryos.

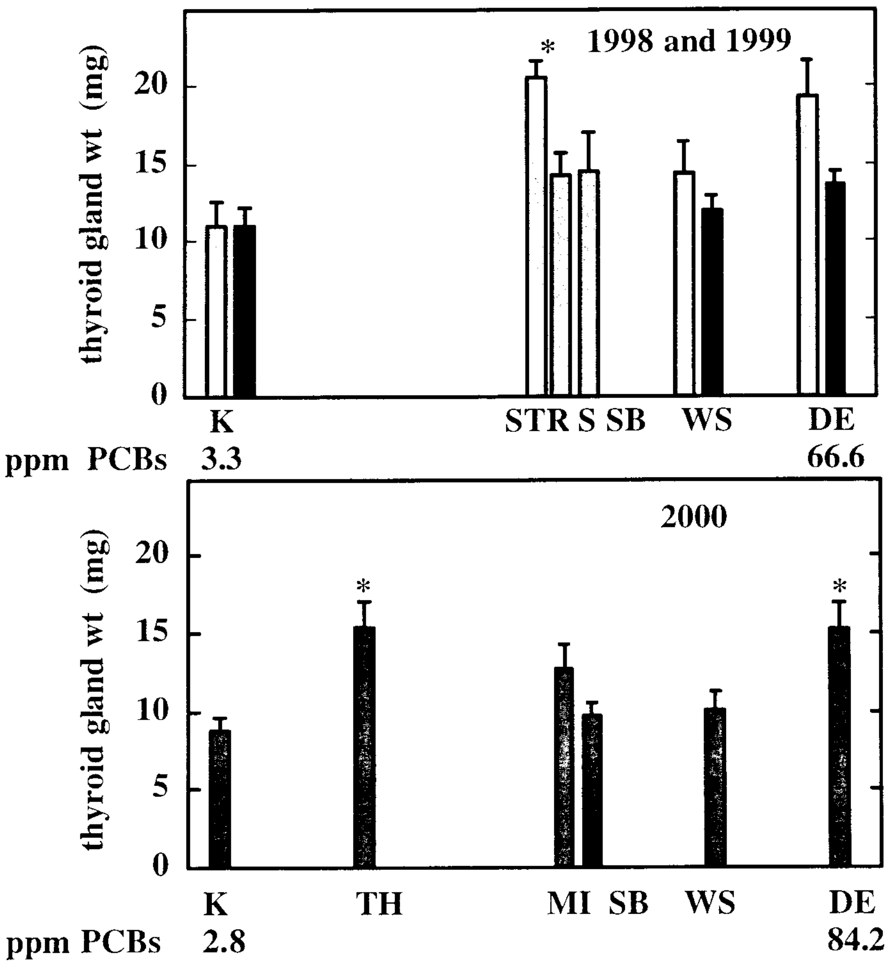

Fig. 14. Thyroid gland weights in pipping herring gull embryos from sites in the Great Lakes. Sites are arranged from low to high mean concentrations of PCBs measured yolk sacs of embryos.

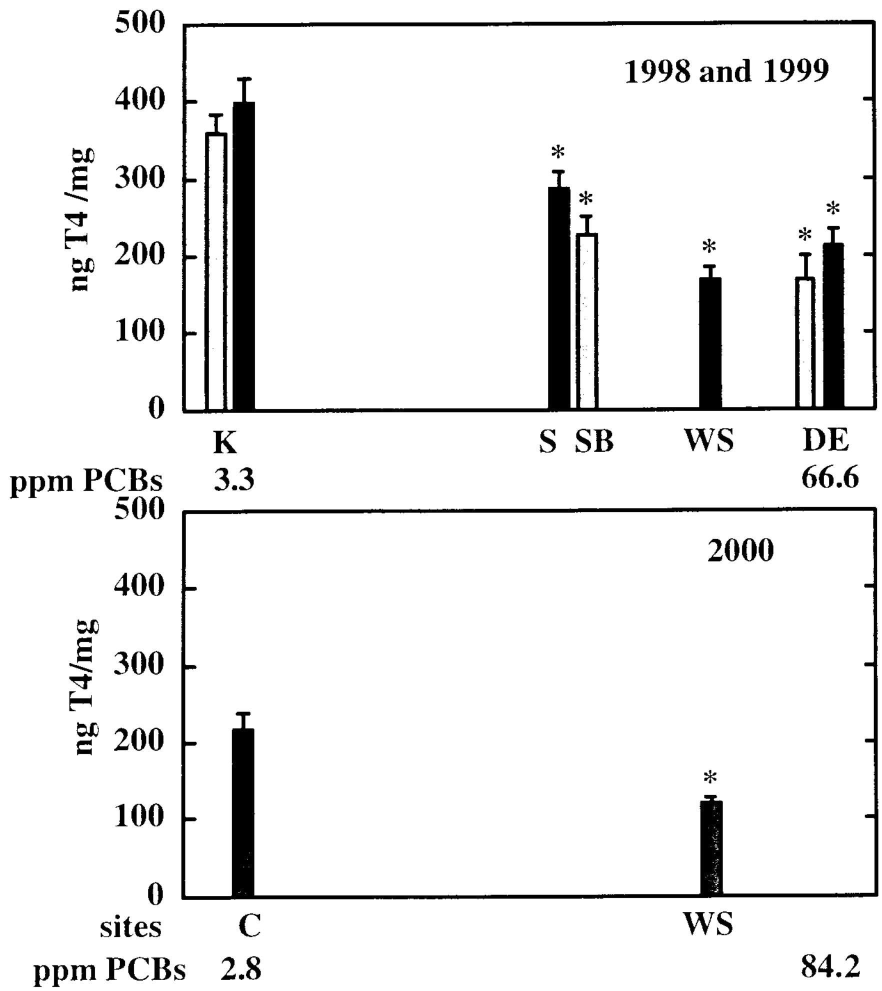

Fig. 15. Thyroid gland T4 content in herring gull prefledglings from sites in the Great Lakes. Sites are arranged from low to high mean concentrations of PCBs measured yolk sacs of embryos.

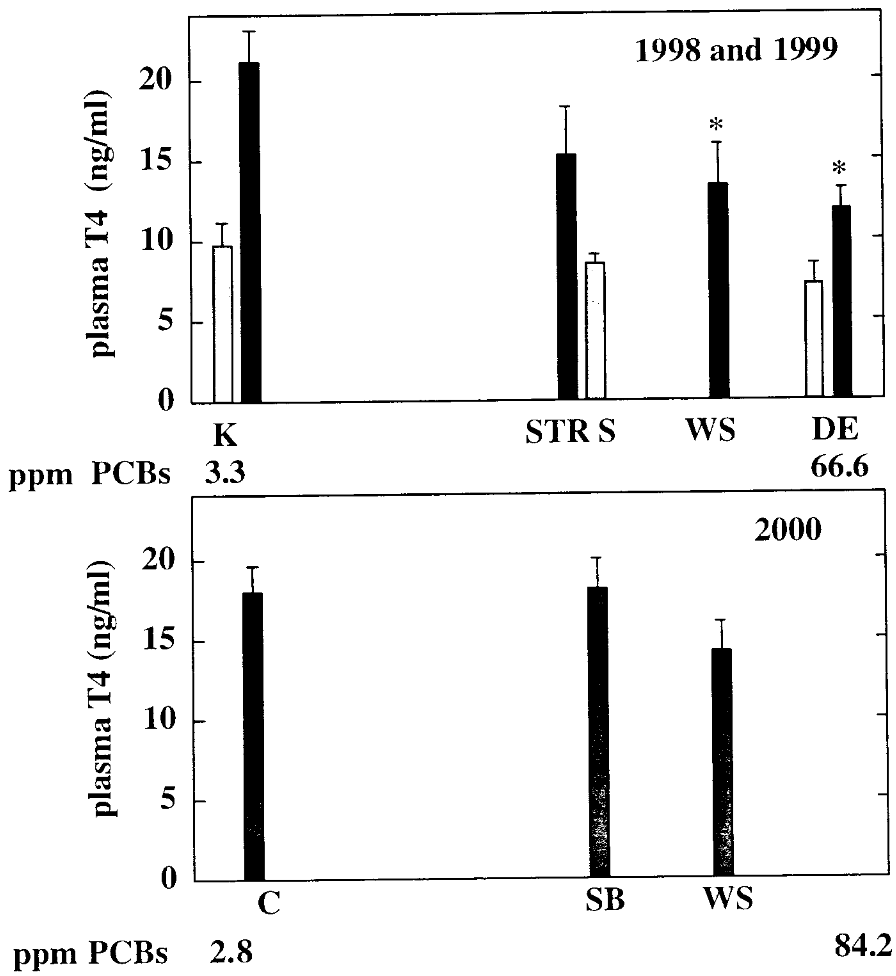

Fig. 16. Plasma T4 in herring gull prefledglings from sites in the Great Lakes. Sites are arranged from low to high mean concentrations of PCBs measured yolk sacs of embryos.

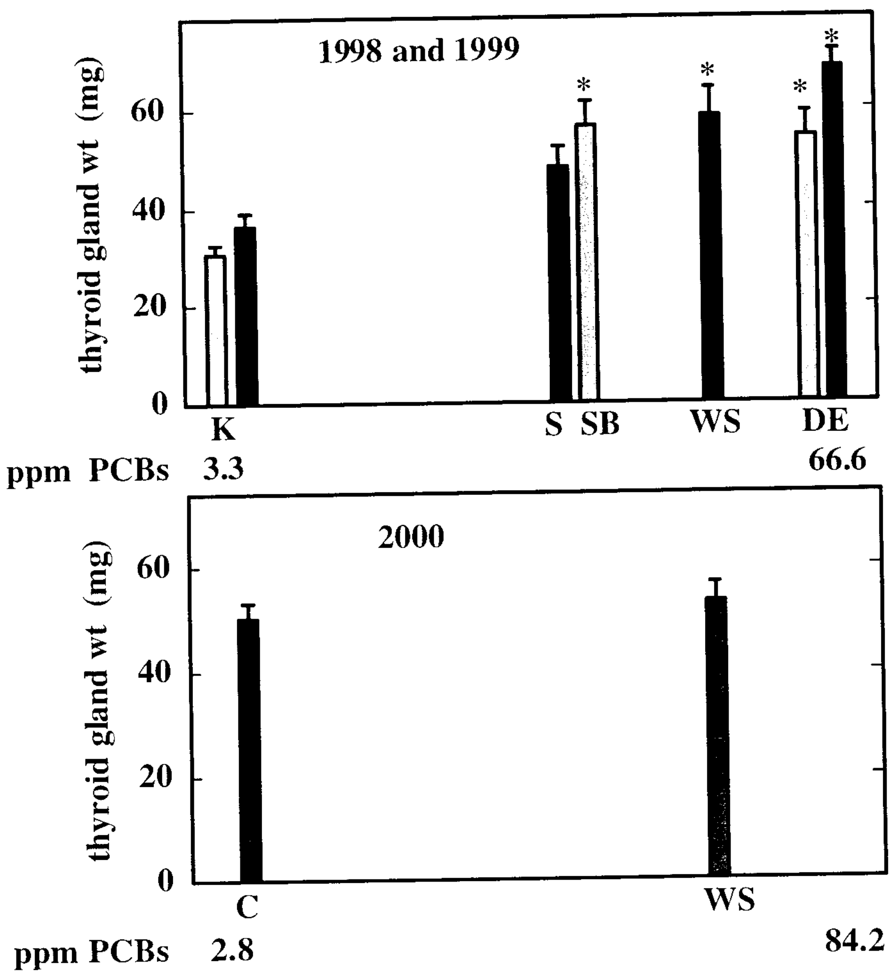

Fig. 17. Thyroid gland weights in herring gull prefledglings from sites in the Great Lakes. Sites are arranged from low to high mean concentrations of PCBs measured yolk sacs of embryos.