Field and Laboratory Studies of the Effects of Polychlorinated Biphenyls and Other Persistent Organic Pollutants on Thyroid Function During Avian Development

Project Scope

Herring gulls (*Larus argentatus*), a fish-eating predator, have been used as a sentinel species to monitor the concentrations and biological effects of environmental contaminants in the Great Lakes for more than 25 years. Past research has focused on mortality, developmental abnormalities, and reproductive effects of chemical pollutants in the lakes. Studies of thyroid histology and developmental effects suggest that thyroid function is disrupted in these gulls. This study examined the thyroid status of developing gulls in the Great Lakes and employed a laboratory model and specific polychlorinated biphenyl (PCB) congeners (PCBs are key contaminants in the lakes) to examine the mechanisms whereby PCBs may disrupt thyroid function in birds (oviparous vertebrates) in comparison to their known mechanisms of disruption in mammals.

The main objectives of this research were to:

- Evaluate the current status of thyroid function in developing Herring gulls (at hatching and prefledgling) from Great Lakes sites with different chemical pollutant profiles; and
- Determine, using laboratory studies on developing chickens (*Gallus domesticus*) if the disruption of avian thyroid function by PCBs is similar mechanistically to that in laboratory mammals.

Project Results and Implications

Field Studies

Herring Gull Field Studies and PCB Analysis.

Gull collection at the Great Lakes sites was done by collaborators, Glen Fox (Contaminant Effects Specialist, Canadian Wildlife Service) and Keith Grasman (Associate Professor of Biological Sciences, Wright State University). The PCB analyses were done by the Canadian National Wildlife Research Centre. Since the 1970s, Herring Gull pipped embryos and chicks have been sampled for PCBs. Pipped embryos are at the end of the 25-day incubation period and are identified by the beak breaking the shell. Pre-fledgling chicks are 28 days old post-hatch and are on the verge of fledgling from the nest.

Grant Title and Principal Investigator

Field and Laboratory Studies of the Effects of Polychlorinated Biphenyls and Other Persistent Organic Pollutants on Thyroid Function During Avian Development

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Key Findings

- Embryonic and prefledgling gulls from polluted areas of the Great Lakes had significantly decreased thyroid function (as determined by lower levels of T4 thyroid hormone) compared to gulls from reference sites.
- Other species (black guillemots and turtles) also showed signs of thyroid hypertrophy and increased liver PCB levels in areas with high-PCB contamination.
- Thyroid gland T4 content was determined to be a more sensitive marker for alterations in thyroid function than plasma hormones or thyroid gland hypertrophy.
- There were no negative effects observed in any measures used to assess thyroid function in chicken embryos exposed to PCB 126 and 77 in the laboratory. This suggests that the mechanisms of toxicity of these PCBs are not through endocrine disruption of thyroid function.
- Activity of the hepatic enzyme UDP-GT (which leads to T4 metabolism and excretion) is not increased appreciably in chicken embryos exposed to PCBs126 and 77. This suggests that these PCB congeners do not affect thyroid function by this mechanism.

Project Period: August 1999 to July 2001

"No cost" extension to July 2003

The sum of 60 PCBs (out of a possible 209 congeners) were measured in yolk sacs from pipped embryos. PCBs are ubiquitous so “low PCB” sites were compared to “high PCB sites”. Table 1 summarizes the PCB levels found in yolk sacs from the reference (low PCB) sites and “high PCB” sites.

Table 1. PCBs in yolk sacs

Site	1998-1999 (parts per million)	2000 (ppm)
Kent Island (Bay of Fundy) Ref. “low PCB”	~1 to 2	~1 to 2
Chantry Island (Lake Huron) Ref. “low PCB”	~3	~3
Toronto Harbor (Lake Ontario) “high PCB”		23.8
Strachan Island (Lake Ontario) “high PCB”	37.7	
Scotch Bonnet (Lake Ontario) “high PCB”	40.2	
Saginaw Bay (Lake Huron) “high PCB”	41.0	54.2
West Sister Island (Lake Erie) “high PCB”	53.4	62.4
Detroit Edison (Lake Erie) “high PCB”	66.6	84.2

Sensitivity of different measures for detecting disruption of thyroid function The investigator’s research (funded by the U.S. Department of Defense, the U.S. Department of Energy, and the U.S. Environmental Protection Agency) compares the sensitivity of a number of thyroid parameters, as well as other growth-related variables that are influenced by thyroid function, to detect thyroid disruption in birds. In all cases, when several thyroid parameters were measured on the group of animals, thyroidal T4 content (per pair of thyroid glands, i.e., the total stored T4 per animal) was the most sensitive measure of alterations in thyroid status, with plasma hormone concentrations and thyroid gland hypertrophy being much less sensitive indicators. Growth-related variables, such as body weight, limb growth, and plumage changes, are very insensitive compared with direct measurements of thyroid function; only thyroid alterations severe enough to result in sustained organismal hypothyroidism are sufficient to cause these types of target organ effects.

Relevance to ORD’s Multi-Year Research Plan

This project contributes to ORD’s MYP long-term goal (2) to determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment.

Herring Gulls in the Great Lakes have been monitored as a sentinel species of environmental health since the late 1970s. Deficiencies in thyroid hormones are associated with developmental abnormalities, most notably in the central nervous system and skeletal structure. The study determined that gulls from areas with high-PCB exposure have altered thyroid function which could compromise their capability to mount responses to altered environmental conditions that would normally increase thyroid hormone secretion. Analysis of the relationships between thyroid function and PCBs compared with other contaminants in the study areas indicate that total PCB concentration is the strongest predictor of thyroid alterations.

The laboratory studies of PCB 126 and 77 effects in chicken embryos suggest that the mechanisms of toxicity of the coplanar PCB congeners tested are not through endocrine disruption of thyroid function. The findings suggest that some of the less toxic PCBs that are present in much higher concentrations than the PCBs chosen for laboratory studies could be responsible for the apparent effects of PCBs on thyroid function in wild gulls.

Thyroid Function in Embryonic and Pre-fledgling Herring Gulls From Great Lakes Sites. Herring gull pipped embryos and pre-fledgling chicks collected from some "high-PCB sites" showed evidence of decreased thyroid function in collections in 1998, 1999, and 2000 compared with those collected at the reference sites at Kent Island in the Bay of Fundy and Chantry Island in Lake Huron. Total PCB concentrations (sum of approximately 60 PCB congeners) in yolk sacs of eggs collected at the high-PCB sites ranged from approximately 7 to 28 times those from eggs collected at the Kent Island reference site.

In herring gull pipped embryos, thyroid gland T4 content (both per mg thyroid and per pair of thyroid glands; the most sensitive indicator of altered thyroid function), was significantly[1] decreased in embryos collected at three of five high-PCB sites in 1998, at one of two in 1999, and at all five high-PCB sites in 2000 compared with birds from the reference sites. In 2000, thyroidal T4 content at four of five high-PCB sites was <10 percent than that in embryos collected at the reference site. Organismal hypothyroidism, as indicated by significantly depressed plasma T4 concentrations, was less frequent than significant decreases in thyroid gland T4 content. That finding indicates that although embryos at some high-PCB sites are able to maintain circulating T4 hormone concentrations, they might be doing so at the expense of depleted thyroid gland hormone stores. Depleted hormone stores suggest that these birds may have a reduced capacity to respond adaptively to environmental challenges that require increases in circulating thyroid hormones.

In pre-fledgling herring gulls, the picture was more complex. Although the thyroidal T4 content per unit weight of gland was depleted significantly in gulls collected at all high-PCB sites in all 3 years, at some sites, the hypothalamic-pituitary-thyroid (HPT) axis response, which results in an increased thyroid weight, compensated for the lower concentration of T4. Therefore, the total hormone content per thyroid gland was not significantly different from that of birds at the reference sites. Thus, pre-fledglings, which have a more mature HPT axis than pipped embryos, appear sometimes to be able to compensate for PCB effects.

Thyroid function in adult herring gulls. Adult herring gulls collected in 2001 at two of the high-PCB areas, which presumably had been exposed to PCBs throughout their lives, had significantly depleted thyroidal T4 content compared with gulls collected at two reference sites.. Other thyroid variables did not differ for gulls collected at the high-PCB and reference sites.

Effects of PCBs on T4 displacement from the hormone-binding protein transthyretin. Some PCB congeners can displace T4 from hormone-binding proteins in herring gull plasma *in vitro*. The percent of free T4 tended to be higher in plasma from pre-fledgling (1998) and adult herring gulls (2001) collected at the high-PCB sites compared to percent free T4 in plasma from gulls collected from the reference sites. Gull egg T4 deposition tended to be lower for gulls from the high-PCB sites compared with reference sites. The 1998 pre-fledglings and the 2001 adults were the only collections for which sufficient plasma was available to measure free hormone levels.

Thyroid effects of PCBs on other species. Black guillemots from a location in Labrador, where the only contaminant present was Aroclor 1260, were compared with individuals from a reference site, a slightly contaminated island site, and a contaminated beach site (birds at the beach site had liver PCB concentrations approximately 11-130 times higher than those at the reference site). In pooled samples, there were no significant differences in thyroid function in birds collected among the sites, presumably because of the high variation in liver PCB concentrations among individuals at the high-PCB sites. At the end of the grant period, analyses of data for individuals had not yet been done.

Turtles hatched from eggs collected at other highly contaminated Areas of Concern in the Great Lakes, but that were reared in the laboratory, had significantly decreased thyroidal T4 content (per pair of thyroid glands) and significant thyroid hypertrophy compared to those from reference sites. These thyroid alterations were correlated with the *in ovo* concentrations of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene), indicating that these contaminants may be contributing to thyroid changes in animals in the Great Lakes (project conducted in collaboration with Kim Fernie, Canadian Wildlife Service).

[1] Analysis of Variance (values of $p<0.05$ were considered significant), followed by post hoc range tests to determine which sampling sites differed from each other.

Laboratory Experiments with Chicken Embryos

Effects of PCBs on thyroid function. The same measures of thyroid function used for the gull studies (i.e., plasma thyroid hormones, thyroid gland weights, and thyroid gland hormone content) were used to assess the effects of PCB-126 (a coplanar, dioxin-like PCB congener; 3,3',4,4',5-pentachlorobiphenyl) on chicken embryos exposed throughout embryonic life. Some experiments also addressed the post-hatch effects of embryonic exposure to PCB 126, and additional experiments used another coplanar congener, PCB 77, instead. A range of PCB 126 doses chosen to demonstrate immunological effects at all doses resulted in essentially no effect on any of the measures used to assess thyroid function in chicken embryos. Additional samples are being analyzed to increase the number of data points. Overall, the work to date suggests that PCB 126, the most toxic and most dioxin-like of the PCBs, has toxic effects (40 percent mortality at the highest dose tested) before any possible thyroid effects are apparent. The results from the PCB-126-dosed eggs were the same for both embryos and chicks allowed to hatched and sampled at several post-hatch times. Altered thyroid function, based on T4 levels, was not observed in embryos or chicks from eggs dosed with PCB 77. In addition to the thyroid variables described above, the activity of 5'-deiodinase (5'-D) in brain tissue in the embryos was measured. This enzyme, which converts T4 to the metabolically active enzyme T3, responds to hypothyroid conditions by increasing activity and "protecting" central nervous system development from thyroid hormone deficiency. The test hypothesis was that this enzyme could be a sensitive indicator of minor alterations in thyroid function. However, consistent changes in 5'-D were not seen after embryonic PCB 126 exposure in chicken embryos, even though chickens generally are considered to be more sensitive to PCBs than most other avian species.

Effects of PCBs on UDP-GT in chicken embryo livers. In laboratory mammals (rats), PCBs cause an increase in activity of the hepatic enzyme, uridine diphosphate glucuronosyl transferase (UDP-GT), which metabolizes T4 and results in a decrease in circulating T4. It is not known if this PCB mechanism of action is valid in avian species. Because there have been essentially no measurements of UDP-GT activity in birds, the researchers modified and validated an enzymatic assay using para-nitrophenol as a substrate for use with avian tissues. The pattern of UDP-GT activity in the chicken was described in order to provide basic information about the developmental patterns of this enzyme in birds. The assay then was used to measure UDP-GT activity in chicken embryos in the PCB 126 studies described above. There was a trend toward increased UDP-GT activity with increasing PCB exposure, but there were no statistically significant effects on thyroid function up to PCB 126 doses that caused 40 percent mortality. Preliminary work was initiated on a UDP-GT assay using labeled T4 as a substrate.

Implications

The laboratory studies of PCB 126 and 77 effects in chicken embryos suggest that the mechanisms of toxicity of the coplanar PCB congeners tested are not through endocrine disruption of thyroid function (which is a known mechanism in mammals). Additional analyses of the other contaminants in the Great Lakes study sites have revealed that of all of the contaminants examined, total PCB concentration is the strongest predictor of thyroid alterations in herring gulls. These findings suggest that some of the less toxic PCBs that are present in much higher concentrations than the PCBs chosen for laboratory studies may be responsible for the effects on thyroid function in gulls observed in the Great Lakes.

Investigators

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For More Information

Principle Investigator's Web Page:
http://www.biol.vt.edu/faculty/mcnabb/

NCER Project Abstract and Reports:

http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/444/report/0