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Toxicological Investigations of Pollutant-Related Effects in Great Lakes Gulls

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Reproductive failure of a number of fish-eating birds was observed on the Great Lakes in the mid-1960s to mid-1970s. The herring gull (*Larus argentatus*) has been used as the primary monitoring species. The low hatching success observed in this species on Lake Ontario in the mid-1970s was due to loss of eggs and failure of eggs to hatch. Egg exchange experiments demonstrated that this was due both to the incubation behavior of adults and to direct embryotoxic effects. Decrease of nest attentiveness was demonstrated using telemetered eggs, but attempts to reproduce the embryonic effects by injection of pollutant mixtures into eggs were not successful.

Reproductive success improved rapidly during the late 1970s and was normal by the end of the decade. Recent studies have focused on cytogenetic and biochemical changes and detailed analytical chemistry of residues. No changes in the rate of sister chromatid exchange over values determined in coastal colonies were observed. Elevation of hepatic aryl hydrocarbon hydroxylase activity, levels of highly carboxylated porphyrins, and changes of thyroid function have been found. The geographic pattern of these changes indicates that they are caused by xenobiotics, but it has not been possible to relate the changes to a specific chemical.

The objectives of this case study are to examine the toxicological approaches used (both field and laboratory experiments and their interaction) and assess their value as tools for wildlife toxicological investigations.

Early Studies

The first study of the possible interaction of pesticides with herring gulls (*Larus argentatus*) was carried out by Keith (*1*) in 1964 as part of wider investigation of organochlorines in the Lake Michigan ecosystem (*2*). Keith found that the overall fledging success was low (0.3–0.4 young per nest). Embryonic mortality was exceptionally high (30–35%), and chick survival was below normal. Residue levels of DDT and its metabolites were measured, but in this pre-PCB era only the measurement of DDE can be considered reliable (*3*).

In Canada the starting point was a visit in 1970 by Gilbertson to several small, artificial islands off Hamilton Harbour, Ontario, where common terns (*Sterna hirundo*) were nesting. Gilbertson (*4*) describes what he saw as follows: "As I wandered about, I soon noticed that something was fundamentally wrong with the colony. While some young of varying age were found in the nests, the eggs in most had failed to hatch. On examining one of these eggs, I found that the young chick had died before it could completely crack open the shell. Several other eggs contained dead embryos. At the edge of a grass tussock, I also noticed an abnormal two-week-old chick, its upper and lower bill crossing over without meeting—a deformity which would result in certain starvation."

Herring Gull as Monitoring Species

Subsequent Canadian Wildlife Service investigations examined double-crested cormorants (*Phalacrocorax auritus*), black-crowned night-herons (*Nycticorax nycticorax*), and common terns, recently summarized elsewhere (*5*), but major effort was focused on the herring gull. The rationale for the use of the herring gull as a monitor of contamination of the Great Lakes has been considered in detail before (*6,7*). In summary, the reasons for the choice were as follows.

1. It feeds at the highest trophic level of the food chains of the Great Lakes.
2. The adult herring gull is a year-round resident of the Great Lakes, with comparatively little movement from lake to lake.
3. The herring gull nests colonially. Colonial birds are probably the only groups of organisms for which it is possible to count the entire breeding population. Collection of eggs and assessment of reproduction are also easier in a colonial species.
4. The herring gull has a wide Holarctic distribution. This allows comparison between contaminant levels, reproductive success, etc., on the Great Lakes with those in coastal and European populations.

Thus the herring gull meets all the requirements of

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an indicator species laid down two decades ago by Moore (*8*), except that gulls cannot be aged readily after their fourth year.

Intensive Phase: 1972–1977

Reproductive Success

Studies of reproductive success and egg residues were initiated by the Canadian Wildlife Service (CWS) in 1972. Herring gulls nesting in the eastern basin of Lake Ontario in 1972 produced only 0.10 to 0.21 chicks per pair (*9*). Studies conducted in 1973 showed that the poor reproductivity was due to a high incidence of egg disappearance and embryonic death (*10*). Reproductive success and egg contamination levels were determined from colonies throughout the Canadian Great Lakes in 1974 and 1975 (*11*). The reproductive success was found to be very low in Lake Ontario (Table 1). The major problems in Lake Ontario colonies were the disappearance of eggs and the failure of embryos to develop. These findings agreed with earlier observations from Lake Michigan (*1*) that it is the hatching success that is affected to the greatest extent. At Scotch Bonnet Island (Lake Ontario) 38.5% of eggs disappeared compared to 2.2% at Chantry Island (Lake Huron) and 10.1% at Granite Island (Lake Superior). On Scotch Bonnet 35.1% embryos failed compared to 5.7% at Chantry Island and 9.4% at Granite Island. Values for the Port Colborne colony (Lake Erie) were intermediate, being 10.1% and 16.7%, respectively.

Keith (*1*) considered that his findings implied that two aspects of embryo mortality were involved, viz., the effect of the residues in the egg upon embryos, and the effect of residues in the adult upon their incubation behavior. Keith's study did not enable him to assess the relative importance of these factors, but egg exchange studies to elucidate this point were carried out as part of the CWS studies.

The presence of a direct embryotoxic effect operating in Lake Ontario in 1974 was demonstrated by artificially incubating eggs collected from colonies in Lakes Ontario and Erie, with eggs from Alberta and New Brunswick serving as controls (*12*). It was found that the percent of eggs that pipped and the percentage of eggs that hatched were significantly lower for eggs from Lake Ontario.

Table 1. Reproductive success of herring gulls in the Great Lakes in 1975.[a]

	Lake Superior	Lake Huron	Lake Erie	Lake Ontario
Eggs per nest	3.01	3.22	3.05	3.07
% hatched	80	72	63	19
Chicks per nest	2.38	2.38	1.93	0.59
% fledged	58	66	73	25
Fledged per nest	1.38	1.48	1.41	0.15

[a] Data from Gilman et al. (*11*).

Egg Exchange Experiments

The basis of an egg exchange experiment is to move eggs from a highly contaminated (dirty) colony and place them under adults in a relatively uncontaminated (clean) colony. The outline of the experiment is shown in Table 2. Additionally it is possible to incubate eggs from both "clean" and "dirty" colonies artificially to examine the effect of the residues on the embryo.

While the basic outline of the egg exchange experiment is simple, the actual logistics are difficult. One potential complicating factor is the effect of transportation on the viability of eggs. This was found not to be a serious problem; in preliminary experiments in which eggs were removed from the nest, transported over a considerable distance and returned, the eggs hatched at a similar rate to eggs that were not moved. Lack of synchronization of laying between clean and dirty colonies was the greatest problem. The results of our egg exchange experiments carried out during the period 1975 to 1977 are given in Table 3.

The results from the 1975 experiment are quite clear-cut: there are major intrinsic and extrinsic effects. Clean adults are unable to hatch dirty eggs and dirty adults are unable to hatch clean eggs. For the worst-case combination, dirty-dirty, the hatch was almost zero. A partial repeat of the experiment in 1976 did not give such a clear picture. While the clean eggs under dirty birds were still significantly lower than the controls of the previous year, the decrease was much less dramatic, the results for the dirty-dirty combination were also less marked than in the previous year. In 1977, no significant differences were noted in any of the categories. What we were in fact seeing was a sudden marked improvement in reproductive success in the herring gull in Lake Ontario. A summary of the temporal trend of reproductive success is given in Table 4. The very low value from Lake Superior in 1981 is considered to be due to food shortage rather than to any toxic chemical

Table 2. Egg exchange experiments.

Adult	Egg	Information obtained
Clean	Clean	Normal reproduction (control)
Clean	Dirty	Effect of residues on embryo (embryotoxic or intrinsic factors)
Dirty	Clean	Effect of residues on adult behavior (behavioral or extrinsic factors)
Dirty	Dirty	Combination of intrinsic and extrinsic factors

Table 3. Summary of egg exchange experiments.

		1975		1976		1977	
Adult	Egg	*n*	% hatched	*n*	% hatched	*n*	% hatched
Clean	Clean	85	86	—	—	93	68
Clean	Dirty	41	10	—	—	48	63
Dirty	Clean	41	7	86	48	58	81
Dirty	Dirty	49	2	43	23	95	70

[a] From Peakall et al. (*6*).

Table 4. Temporal trends in reproductive success of herring gulls in the Great Lakes.[a]

Location	Average number of 21-day-old chicks per nest					
	1972	1975	1976	1977	1978	1981
Lake Ontario, Scotch Bonnet	0.12	0.15		1.10	1.01	2.13
Lake Erie, Port Colborne		0.65	0.79		1.45	
Lake Huron, Chantry Island		1.48		1.12	1.40	2.16
Lake Superior, Granite Island		1.32			1.12	0.46

[a] Data of Mineau et al. (*13*).

effect. Organochlorine residue levels were decreasing rapidly at this time (*13,14*).

Behavioral Effects

The egg exchange experiments of 1975 indicated the presence of both intrinsic and extrinsic factors in the reproductive failure of the herring gull. The nesting behavior of the gulls at the failing Scotch Bonnet colony on Lake Ontario and a coastal colony (Kent Island, New Brunswick) was examined by Fox and co-workers (*15*). These studies were made in 1975 and 1976. They found that the minimum flushing distance of adults from their eggs was much greater on dirty than on clean colonies, that the number of swoops by adults on observers was decreased, and strikes did not occur. By contrast, Ludwig and Tomoff (*16*) noted that herring gulls in the failing colonies on Lake Michigan were "exceptionally aggressive towards us and each other," and these workers considered that this aggressive behavior might be causing the poor reproductive success noted in Lake Michigan colonies. It is possible that nonpollutant-related factors, such as colony size and prior human disturbance patterns, are involved in these differences.

Detailed studies of incubation behavior were made by using telemetered eggs (*15*). These gave information on the core temperature, surface temperature, and intensity of light falling on the small pole of a wax-filled herring gull egg (*17*). Comparisons were made between the failing colony at Scotch Bonnet, Lake Ontario, and the successful coastal colony at Kent Island, New Brunswick. Examination of the distribution of surface egg temperatures from the two colonies shows that the Lake Ontario values were significantly skewed toward lower readings. In the case of the Kent Island colony, no values below physiological zero for development of the embryo (27°C) were recorded, whereas for Lake Ontario a considerable number of values in the range of 13 to 26°C were found (*15*).

A similar pattern was also found when successful and unsuccessful nests in the Lake Ontario colony were compared. The minimum temperature recorded from successful nests was 25°C, whereas temperatures ranged down to 13°C for unsuccessful nests. Decreases in nest attentiveness were also found (Table 5). While the changes noted are numerically small, the predatory habits of neighboring gulls make them biologically important.

Table 5A. Nest attentiveness of herring gulls: comparison of Lake Ontario and Kent Island colonies.[a]

	Day		Night	
	Lake Ontario	Kent Island	Lake Ontario	Kent Island
Time spent incubating, %	96.2	98.9	97.6	99.2
Mean length of absence, min	3.4	1.2	7.4	1.9

[a] Data of Fox et al. (*15*).

Table 5B. Nest attentiveness of herring gulls: comparison of successful and failing nests on Lake Ontario.[a]

	Successful	Failing	Successful	Failing
Time spent incubating, %	97.7	92.3	95.9	96.9
Mean length of absence, min	2.4	6.4	10	29

[a] Data of Fox et al. (*15*).

Egg Injection Experiments

Egg injection experiments were carried out at the large (~20,000 pair) colony of herring gulls at Kent Island (*18*). It was decided to use fresh eggs (0–2 days), despite the fact that mortality due to injection is higher in newly laid eggs than those that are several days old. This enables the effect of pollutants to be studied during the critical early development of the embryo. This increased mortality was compensated for by having a large sample size (~100 eggs) per group. Since any study of this type is a considerable undertaking, several experiments were run simultaneously. We were concerned that any synthetic mixutre would not exactly match the chemicals as they occurred in the field situation. To overcome this problem, herring gull eggs collected on Lake Ontario were extracted for re-injection. There were four "extraction" groups, viz., total Lake Ontario, nonpolar Lake Ontario (PCBs, DDE, mirex), polar Lake Ontario (cyclodienes, hexachlorobenzene), and an extract control. Additionally four "synthetic" groups were used, viz., mixtures equivalent to Lake Ontario, Lake Ontario without PCBs, Lake Huron, and a solvent control group. The chemical analysis of these eight groups is given in Gilman et al. (*18*). In each three egg clutch, two eggs were injected with a different experimental solution and the remaining egg was either uninjected or an injected control. One obvious question in an experiment such as this is whether the uptake of injected contaminants into the embryo is the same as that which occurs with naturally deposited contaminants. To check this, gull eggs were collected at 7, 14, 21, and 28 days of incubation at Granite Island, Lake Superior, and levels of organochlorines (OCs) in yolk and the embryo determined and compared to a group of eggs injected at Kent Island. No significant differences in the uptake were found (*18*).

The results of the massive experiment (the fate of

nearly 1200 eggs was followed) is quickly summaried; no significant differences were seen between any groups. It was concluded that either the intrinsic factor was not extracted or that the effects of ingested toxic chemicals are manifest before the egg is laid.

More recently, egg injection studies under artificial incubation conditions were carried out to determine the effect of mirex and HCB (*19*). No significant effects of mirex were determined up to the highest dose used (5 ppm), but 75% mortality was caused by HCB at 5 ppm and the embryonic LD_{50} was calculated to be 4.3 ppm. Values of HCB in this range were found in herring gull eggs from Lake Ontario in 1971 and 1972, but levels from 1974 onwards were well below this level (*20*).

Residue levels of OCs in eggs were measured in 1974 and 1975. The values of the most significant residues are given in Table 6. The most important contaminant discovered after these experiments were performed are the tetrachlorodioxins. The possible impact of these highly toxic compounds will be discussed later, but it would have been expected that TCDD would be present in the total and polar extracts of the Lake Ontario eggs.

The final result at the end of the intensive phase of investigation (1972–1977) was that some behavioral abnormalities had been demonstrated, but injection of the toxicants back into eggs did not correlate with the findings of the egg exchange experiments. The reproductive rate was improving rapidly over this period and this tended to confound the results.

Levels of OCs were decreasing markedly during the period 1974–1980 (*13,14*). In Lake Ontario the levels of PCBs in herring gull eggs decreased almost linearly over this period from 150 ppm in 1974 to 60 ppm in 1980; the corresponding figures for DDE were 22 ppm and 8 ppm (*14*). In contrast, during the first few years of the eighties the levels have tended to remain constant or even increase slightly (*14*). Further, as Gilbertson demonstrated as long ago as 1973 (*9*), the levels of the various OCs are strongly correlated with each other, making assigning of specific effects with specific chemicals very difficult.

Monitoring Phase, Post 1977

After the intensive phase our studies have continued since there are still concerns over the health of the Great Lakes. These studies can be listed under four main headings: (1) incidence of congenital anomalies and cytogenetic studies to assess the occurrence of teratogenic and genotoxic agents in the environment; (2) biochemical studies to identify parameters with potential as monitors of biological effects of pollutants; (3) detailed analytical studies to identify additional contaminants and modeling of the pathways of known contaminants; (4) long-term trend monitoring of the chemical residues of OCs in gull eggs.

Table 6. Organochlorine residue levels in herring gull eggs in 1974 and 1975 (part per million, wet weight).

	OC residues, ppm				
	Lake Superior	Lake Huron	Lake Erie	Lake Ontario	Lake Michigan
DDE	19	14	7	23	32
Mirex	0.7	0.6	0.3	5.1	Trace
PCB	60	52	66	142	92
HCB				1.7	
TCDD (ppb)				0.6	

[a] Data of Gilman et al. (*11*).

Congenital Anomalies and Cytogenetic Studies

A program to determine the incidence of congenital anomalies in colonial fish-eating birds was set up by CWS as a biological monitor of teratogens in the aquatic environment (*21*). The program is largely dependent on amateur bird-banders and the existence of a central agency for handling banding returns. The choice of fish-eating birds is based on the fact that they share a common food resource with man and concentrate lipophilic contaminants to a very marked extent. Many of the species nest in large colonies which are re-occupied year after year. They are banded in large numbers so that a considerable data base is available. (Circa two million individuals of the species used in the program were banded in North America over the period 1955–1980.) It was found that the prevalence of anomalies in herring gull chicks was 100- to 200-fold above the background level (1 in 20,000) in Lake Ontario colonies in the period 1971–1975 (*14*). The incidence of anomalies has now returned to background levels here, although elevated incidence has been recently reported (*22*) for the double-crested cormorant from Lake Michigan.

Sister chromatid exchange (SCE) rates were measured in 7-day herring gull embryos from eggs collected in 1981 from five colonies within the Great Lakes basin and one Atlantic coast colony (*23*). Eggs were collected and incubated artificially for 7 days before SCE determinations were made. The average rate of SCE/chromosome for the six colonies ranged from 0.069 to 0.101, but no significant differences were found among any of the colonies. Additional testing was carried out (*24*) by extracting homogenized eggs and testing them in the Salmonella/mammalian microsome assay for induction of point mutations and in the Chinese hamster ovary cell for the induction of sister chromatid exchanges and chromosome aberrations. None of the extracts were mutagenic in Salmonella. In the experiments with mammalian cells, all of the extracts, including the control, gave positive responses to both SCE and chromosome aberrations. No relationship could be established between contaminant levels and the magnitude of the response. It seems unlikely that a contaminant would be present in the control eggs (from Kent Island) at a level comparable with those from the Great Lakes. It is more likely that a lipophilic compound(s) of biological origin, perhaps carotenes, caused the effects seen.

Biochemical Indicators

Heaptic aryl hydrocarbon hydroxylase (AHH) levels were measured in 20- and 25-day old embryos (*25*) from

the same colonies that had been used for the genetic studies. The 25-day embryos were found to show a greater response than the 20-day embryos. Significantly elevated values were found in embryos from Presqu'ile (Lake Ontario) and Saginaw Bay (Lake Huron) compared to the control colony (Kent Island). No significant difference was found between the controls and those collected from Lake Superior and Chantry Island (Lake Huron). A good correlation was obtained between the level of AHH activity and the concentration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin present in the eggs. No such correlation was found with PCBs and DDE. Subsequently, studies were made on 25-day embryos measuring the levels of other mixed function oxidase enzymes (*19*). No significant differences were found for either ethoxyresorufin *o*-dealkylase (EROD) or aminopyrene-*N*-demethylase between the Great Lakes and the control colony. The level of aniline hydroxylase was significantly lower in seven of the eight Great Lake colonies, and this was negatively correlated with mirex levels. Regrettably, AHH levels were not measured in this study so that the differences found, especially those between EROD and AHH, cannot be directly examined.

The histopathology of the thyroids of adult herring gulls from the Great Lakes has been examined and compared with those from coastal colonies (*26*). Increases of relative mass, epithelial hyperplasia, and decreases of follicular diameter, epithelial area, and colloidal vacuolation were noted in birds collected from the Great Lakes as compared to coastal birds. These findings are similar to those previously reported for salmon (*Oncorhynchus* spp.) from the Great Lakes (*27*). The geographical distribution of the changes found in avian thyroid histopathology is complex, and it is not clear to what extent chemical factors are superimposed on the overall iodine deficiency of the Great Lakes.

An ultrasensitive method for the determination of the tissue porphyrin pattern has been developed recently (*28*) as part of the CWS program to establish biological indicators of contamination. Earlier studies (*12*) had indicated the porphyria might have been one of the causes of herring gull reproductive failure in the mid-1970s, but these studies were handicapped by the lack of good analytical techniques for porphyrins. Using this new methodology based on high-pressure liquid chromatography, it was possible to detect the highly carboxylated porphyrins (HCPs) down to 4 to 10 pmole/g. In livers of herring gulls from marine colonies HCP levels were 5 to 24 pmole/g and similar levels were found in a variety of other species with differing diets collected from relatively uncontaminated areas. In contrast, the mean values for herring gulls collected on the Great Lakes basin in the interval 1974–1985 varied from 2 to 38 times the median of those collected from the Atlantic coast (*29*). It was found to be possible to carry out analysis on samples that had been stored in the National Tissue Bank. The highest median levels were obtained from specimens collected from eastern Lake Ontario in 1974 and in recent material from Saginaw Bay (Lake Huron), Green Bay (Lake Michigan), and Hamilton Harbor (Lake Ontario). The levels in 1974 from material collected from Lake Erie and Lake Ontario averaged approximately twice those found in 1985. The geographical and temporal distribution of HCP levels provides strong support for a chemical etiology, although it is not possible to correlate the levels with specific chemical residue levels.

The levels of hepatic retinol and retinyl palmitate were measured for gulls from New Brunswick and from Great Lake colonies (*30*). The highest mean values of both compounds were found in the New Brunswick gulls and the lowest mean values in those from Lake Ontario. There was a wide range of individual values from all four sites (New Brunswick, Lake Ontario, Lake Michigan, and Lake Superior) that were studied; for example, the retinol values for 20 specimens from New Brunswick ranged from 43 to 2012 μg/g. Nevertheless, the levels were significantly lower in birds from the Great Lakes compared to those from the coast, and there were also significant differences between colonies in the Great Lakes. Experimental studies exposing ring doves (*Streptopelia risoria*) to a dioxin analog (3,4,3′,4′-tetrachlorobiphenyl) demonstrated an inverse relationship between retinol concentration and AHH activity. Since AHH activity was not determined in gulls, it was not possible to examine for this correlation in the target species.

Recent Analytical Studies/Tissue Banks

Analytical techniques have improved greatly in recent years. It is now possible to quantify OC residues in biological tissue in the ppt range and to resolve the individual isomers of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs).

A method to determine the levels of tetrachlorodibenzo-*p*-dioxins (TCDDs) in herring gull eggs was reported in 1982 by Norstrom and co-workers (*31*). This technique was capable of determining levels of this highly toxic material down to <10 ng/kg. Subsequently this technique was used to determine the temporal trends of TCDDs in material stored in our National Tissue Bank. It was found that levels in herring gull eggs from Lake Ontario colonies had decreased from ca. 1000 ppt to ca. 100 ppt over the period 1971–1980 (*32*). The geographic variation of TCDD and other PCDDs has shown that the highest residues are found in Green Bay (Lake Michigan) and Saginaw Bay (Lake Huron) (*33*). Pattern recognition techniques, based on multivariate statistical methods, have been applied to characterize these complex environmental residues (*33*). Application of this technique to PCDD, PCDF, and PCB residues in Great Lakes fish and gull eggs has been made (*34*).

Tissue banks are an important component of both monitoring programs and detailed analytical investigations of residues. The preservation of archived material enables temporal trends to be established without waiting many years. Material from the CWS National

Tissue Bank has been used for several such analyses in addition to the dioxin example (*32*) already referred to. We have been able to determine trends in OCs in a number of seabird species and in polar bears (*Ursus maritimus*). These studies and the details of the operational procedures of the tissue bank have been given by Elliott (*35,36*).

Long-Term Trend Analysis of Residue Levels

One of the major studies undertaken under the Canada–U.S. Great Lakes Water Quality Agreements has been the measurement of OC residue levels in herring gull eggs. No attempt is made here to review the data collected in these studies in any detail. Under this program, two colonies per lake were designated monitoring colonies, and collections were made annually. The findings from this program over the period 1974–1979 have been recently reviewed (*13*). Significant decreases of many OCs in most colonies have occurred during this period. The calculated half-life for PCB ranged from 3 to 10 years, for DDE from 2 to 4 years, and for mirex from 1.5 to 4 years. Recent data have shown that these declines in residue levels have largely bottomed out and some increases have been noted (*14*).

Herring gull eggs collected over the period 1978–1982 from colonies in the Detroit River, Niagara River, and Saginaw Bay have been examined for OCs (*37*), and studies on additional colonies in Lake Erie have been made (*38*). Discriminant function analysis of the OC load of gull eggs from Fighting Island on the Detroit River (*37*) indicates that herring gulls there are accumulating OCs to levels that are significantly different from either Lake Huron or Lake Erie. This finding suggests that there are primary sources along the Detroit River itself. Similarly, the levels from Saginaw Bay are significantly higher than Lake Huron and did not decline over the period studied. These data indicate that the herring gull has value as an indicator of regional chemical contamination within the Great Lakes.

Toxicological Assessment of Complex Mixtures

The major toxicological problem that has to be addressed—one common to most real world situations—is the handling of the effects of complex mixtures. In the Great Lakes, the situation reaches extreme complexity. Even for the OCs alone the situation is complex. While the ratios of the various OCs in various parts of the Great Lakes do vary (mirex levels are highest in Lake Ontario, whereas DDE/PCB ratio is highest in Lake Superior) there are strong cross-correlations between OC residue levels. This makes it difficult to isolate the effect due to any specific compound. Despite the use of more powerful statistical methods in recent years, it is rarely possible to demonstrate that a specific effect has a specific cause. Usually one cannot get further than to demonstrate that the effect is greater in areas of high overall pollutant load.

Despite the efforts of recent years to understand the toxicological significance of complex mixtures, comparatively little progress has been made (*39*). In most cases, complex mixtures are assessed by adding them to a test system as if they were single substances (*40*). The approach of successively adding in components has, for obvious reasons, rarely progressed beyond tertiary mixtures. Nevertheless, these studies suggest that the effects of mixtures are normally additive, rather than synergistic (*39*).

Summary and Conclusions

The marked reproductive impairment noted in the early to mid 1970s was caused both by effects on the adults and direct embryotoxic effects. Reproductive success increased rapidly in the late 1970s and is now normal.

Biochemical indicators—mixed function oxidases, thyroid function, porphyrin levels—show that xenobiotics are still present in sufficient quantity to cause significant changes. While there is no evidence that these changes cause adverse health effects to the organism, these changes can be used as a sensitive monitor of the presence of biologically significant concentration of contaminants in the ecosystem. The geographical and temporal distribution of these effects indicates that they are caused by xenobiotics, but it has rarely been possible to ascribe the effects to any specific chemical.

These studies have been a team effort involving a considerable number of investigators. Many of these are cited in the list of references. Specific acknowledgement should also be given to J. A. Keith, who has been involved in these studies since the beginning when he worked as an investigator and who as a director of CWS has continued to give his unswerving support; M. Gilbertson, whose interest has never flagged since he made his early observations 16 years ago; A. P. Gilman, one of the chief investigators during the intensive phase of the study; and R. J. Norstrom, who headed the analyticial chemistry studies throughout the project.

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