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DEFORMITIES IN BIRDS OF THE GREAT LAKES REGION ASSIGNING CAUSALITY

Ecotoxicology and especially wildlife toxicology are relatively new fields of endeavor (1). Cause-effect linkages between the exposure of wildlife to synthetic compounds and observed population-level effects remain difficult to establish (2). Although there are ancient records

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of human-caused episodes of toxicological effects in populations of wildlife species, only since World War II—when the use of synthetic, halogenated hydrocarbons became widespread—have large-scale, chemically induced wildlife epizootics (epidemics) occurred. Limited knowledge of the basic biochemistry, physiology, and natural histories of wildlife species has prevented a complete understanding of the effects of contaminants on wildlife.

The study of effects of toxic chemicals on wildlife populations is limited by the complexity of many species interacting with one another and their natural habitats, human-caused physical changes to their environment, and the effects of chemicals. This situation has been exacerbated by a lack of sufficiently sensitive and discriminatory instrumental, analytical techniques and authentic standards for all of the compounds that occur in wildlife. However, multidisciplinary research teams of experts in environmental and analytical chemistry, toxicology, biochemistry, and pathology are beginning to provide a comprehensive picture of the effects of trace concentrations of toxic, synthetic hydrocarbons on wildlife species (2, 3).

Historically the colonial, fish-eating water birds (CFEWB) of the Great Lakes have been exposed to many toxic, synthetic, halogenated compounds (3–8). Eggs of Great Lakes birds contain polychlorinated, diaromatic hydrocarbons (PCDH) such as organochlorine insecticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) at sufficiently great concentrations to cause adverse effects on the birds and their chicks (9). These exposures have been correlated with adverse effects on the reproductive potential of several bird species (10), which in turn caused decreases in populations (11, 12). The most dramatic effect on reproductive performance of wild birds was eggshell thinning, caused primarily by DDE, a degradation product of the insecticide DDT (6, 13–15).

Since the restriction of the manufacture and use of some of the PCDH, concentrations of these compounds have decreased in the tissues of birds, their eggs (7, 16), and their food (16). Concomitant with these decreases have been increases in the populations of most of the CFEWB on the Great Lakes. Concentrations of DDE in most species at most areas of the Great Lakes have now decreased below those that cause critical degrees of eggshell thinning. Although this trend is encouraging, recent data suggest that the rate of decrease of concentrations of both PCBs and DDE have now slowed because of internal recycling and continued inputs, primarily from atmospheric deposition (17, 18). Concentrations of PCDHs cannot be expected to decrease further very rapidly.

While concentrations of the routinely measured contaminants have been declining, effects such as embryo lethality and birth defects have persisted in CFEWB in several areas of the Great Lakes (10, 19, 20). Reproductive productivity of bald eagles that eat fishes of the Great Lakes is also lower than that of eagles in less-contaminated regions and in healthy, expanding populations (21, 22). In 1993, four eaglets with deformed bills or feet were observed by our research team.

It was deemed important to determine the causes of the observed effects so that appropriate control measures and management decisions relative to rehabilitation of wildlife populations, and advisories about human consumption of fishes, could be developed. A series of studies was undertaken by our research teams to determine the causes of these effects. It had been suggested that the adverse effects observed were not related to the concentrations of contaminants in the environment.



There were several possible causes to investigate (see box). The suite of observed effects did not seem to be explained by disease or nutrition. Because the bird populations had been greatly reduced and some species almost completely extirpated from the Great Lakes region by the effects of DDE, populations were increasing from a few individuals. It had been suggested that the decreased viability of eggs and abnormally great number of birth defects could have been caused by low genetic diversity ("founder effect").

The effects might have been the result of exposure to newer pesticides that could have effects at very small concentrations and leave little or no trace in the eggs of afflicted birds. Such effects have not been observed in laboratory or field trials with these insecticides, so they did not seem to be a likely cause (1). As we began our investigations in 1985, one possibility seemed to be some unidentified compound or compounds, perhaps from the pulp and paper industry, which is known to release a large number of poorly characterized and seldom identified compounds (23).

Complex mixtures

It is difficult to understand or predict the effects of complex environmental mixtures on biota, in part because their concentrations change as a function of space and time (24). Thus, the mixture to which organisms are exposed at one time or location may be different from that to which they are exposed at other times or locations (25). Furthermore, the relative concentrations of the various PCDH congeners is different from one trophic level to another (26). These differences are caused by environmental weathering and the sorting of compounds, based on their solubilities, volatilities, and rates of degradation.

Additionally, metabolic and pharmacokinetic processes as well as food vary among bird species, causing changes in the composition of the chemical mixtures to which birds are exposed. These processes result in mixtures in the environment that not only change spatially and temporally, but are different from the original mixtures released into the environment. Thus, it is nearly impossible to use the results of studies of dose-response relationships of technical mixtures, determined under laboratory conditions, to predict effects in wildlife in their natural environment.

Adverse effects in embryos and chicks of colonial, fish-eating water birds of the Great Lakes and proposed causes

Effects

- Eggshell thinning
- Deformities
- Tumors
- Behavioral changes
- Immune suppression
- Edema
- Cardiovascular hemorrhage
- Hormonal changes
- Enzyme induction $P_{450}1A1$, $P_{450}1A2$ and $P_{450}2B1$
- Metabolic changes, wasting syndrome
- Depletion of vitamin A
- Porphyria

Deformities

- Crossed bill
- Clubbed foot
- Hip dysplasia
- Dwarf appendages
- Ascites/edema
- Eye
- Brain
- Skull bones
- Gastroschisis

Possible causes

Nutritional deficiencies due to changes to prey base	Disease, such as viral infections
New generation pesticides	Genetic inbreeding
Continued effects of traditional contaminants	Old, persistent pesticides
	Unidentified chemicals, pulp and paper industry

The study of the effects of PCDH on fish and wildlife was limited for two decades by the impossibility of assessing the toxicity of constantly changing mixtures. Furthermore, the interpretation of toxic effects was based on laboratory studies with single compounds or technical mixtures, which are different from those to which wildlife is exposed.

Hydrocarbons

The suite of symptoms that had been observed to occur in populations of wild CFEWB (see box) is similar to those observed during laboratory exposures to various halogenated hydrocarbons. These symptoms are most similar to those caused by chlorinated dibenzo-*p*-dioxins and structurally similar compounds (8, 27-29). In certain species of CFEWB, in some areas, these symptoms have been attributed to PCDH, which are biomagnified by these birds and deposited into their eggs (3, 10, 19, 24-26, 30, 31).

The suite of reproductive anomalies observed includes specific biochemical alterations such as the induction of cytochrome P_{450} mixed-function monooxygenase enzymes (32, 34), depletion of hepatic reserves of retinoids and vitamin A (33), porphyria (35, 36), and wasting syndrome (20, 37). In fact, the observed symptoms are similar to chick edema disease, which can be caused by 2,3,7,8-substituted PCDD and PCDF and structurally similar PCB congeners (3, 20, 27, 28-40). This suite of symptoms has been named the Great

Lakes Embryo Mortality Edema and Deformities Syndrome (3).

2,3,7,8-TCDD equivalents

Recently, greater understanding of the mechanisms of toxic action of the PCDH has made it possible to apply quantitative structure-toxicity relationships to integrate effects of dioxin-like congeners that bind to the aromatic hydrocarbon receptor (Ah-r). It is through this receptor that most of the congeners' toxic effects are hypothesized to be mediated (41-43). With this approach it is possible to obtain better correlations between observed effects and 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin equivalents (TEQ, also referred to as TCDD-EQ) than could be obtained for single PCDH congeners of PCDH (25, 44).

The power of individual PCDH congeners to cause toxic effects can be compared through the use of toxic equivalency factors (TEFs) to that of the most toxic PCDH, 2,3,7,8-TCDD. TEFs can be based on several endpoints, including lethality, deformities, or enzyme induction (24, 29, 41, 44, 45). TEFs can then be used to calculate the Ah-r activity contributed by individual congeners in a mixture. These can be summed and expressed as a total equivalent concentration of 2,3,7,8-TCDD (22).

This additive model seems to be effective in predicting the potency to cause enzyme induction, embryo lethality, or teratogenicity (41, 44). However, this simple additive model does not take into account in-

teractions among Ah-active congeners or among Ah-active and non-Ah-active congeners and other toxic synthetic halogenated compounds that are also present in CFEWB eggs (22, 24, 25, 31).

As an alternative to the additive model, which uses TEFs and instrumentally determined concentrations of individual congeners to determine TEQs, an in vitro bioassay can be used to determine the biological potency of extracts that contain complex mixtures of PCDH congeners (22, 46–48). The assay utilizes the capacity of extracts of tissues, which contain PCDH, to induce specific cytochrome P₄₅₀-requiring mixed function oxygenase enzymes in cultured rat hepatoma cells (H4IIE). The ability to induce ethoxresorufin-o-deethylase (EROD) is correlated with the affinity of PCDH for the Ah-r (44).

It has also been demonstrated that the potency for enzyme induction by the individual congeners is correlated with the power of the congeners to cause weight loss and thymic atrophy in mammals (41) and deformities in and lethality of bird embryos (29, 38, 49). Therefore, induction of enzymes under the control of the CYP1A locus in H4IIE cells serves as an effective, integrative measure of the relative toxic potency of complex mixtures, which contain PCDH (47, 50). Other cell lines or inducible endpoints that are under control of the CYP1A locus can be used as detector systems. The H4IIE system was selected because it expressed little P₄₅₀-1A1 activity constitutively, but is highly inducible.

The H4IIE bioassay has advantages over the use of standard instrumental techniques of analytical chemistry because it measures the potency of exposure close to the site of action by measuring a biological response. The bioassay is quicker and less technically demanding than congener-specific chemical analysis of complex environmental PCDH mixtures. Also, the H4IIE bioassay is useful as a data reduction tool because it integrates the potency of a mixture of PCDH congeners of widely varying toxicities as well as their interactions with other synthetic, organic chemicals (41) and obviates the need for authentic standards for all of the PCDH.

The H4IIE bioassay does not provide information on the cause of the effects and should be used in concert with instrumental analyses. The H4IIE bioassay is particularly useful for determining whether all

of the TEQs in an extract have been accounted for and whether there are nonadditive interactions among congeners. The H4IIE bioassay can also be used with fractionation schemes to guide where to apply more rigorous chemical analyses. Furthermore, other cell lines from specific species of interest can be used, and each of these cell lines can be genetically engineered to include specific reporter genes, which can result in more sensitive assays.

Symptoms of PCDH intoxication

If planar PCDH are considered causal agents in embryo lethality and teratogenesis in CFEWB of the Great Lakes, the question arises, Why are the dioxin-like effects being observed now and why weren't they apparent 20 years ago when the concentrations of planar PCDH in bird eggs and fish were 10 to 100 times greater? Our working hypothesis is that the more subtle symptoms of PCDH poisoning were previously masked by the effects of organochlorine pesticides, notably DDT and its metabolite, DDE. The eggshell thinning caused by these compounds made the eggs of the CFEWB too thin to survive incubation. The eggs could not survive long enough for the effects of the PCDH to be expressed, much less be observed. Furthermore, field biologists were not looking for embryonic effects until the 1980s.

The next consideration in the determination of a causal relationship is to correlate the observed symptoms with the suspected causal agent. For our work this came in two steps: first, to determine the likelihood that PCDH were occurring at sufficient concentrations to cause the observed symptoms in CFEWB and second, to determine whether there was a gradient of magnitude of the symptoms that was correlated with the concentrations of PCDH in different locations of the Great Lakes. In other words, were the PCDH greater than the threshold required to cause symptoms and could a dose-response relationship be demonstrated under field conditions?

As for the first step, we felt that the concentrations of dioxin-like compounds in the CFEWB of the Great Lakes were sufficiently great to cause symptoms of toxicity if the birds displayed sensitivities to the PCDH that were similar to those of the model animals studied in the laboratory (Figure 1). The concentrations of 2,3,7,8-TCDD were, on average, approximately 10 ng/kg,

ww (pptr), which would be at the lower end of the effects range. However, the concentrations of TEQ in eggs of CFEWB as measured by the H4IIE bioassay were found to be 100–1000 ng/kg (24, 26, 47, 48), well within the range of effective concentrations to cause the observed symptoms.

As for a gradient of symptoms, numerous studies have demonstrated that CFEWB displayed symptoms of varying severity and that the severity was correlated with concentrations of PCDH (1, 10). Previously, work was restricted to comparing results from "reference locations" to one or two locations known to be contaminated. It wasn't until our work with double-crested cormorants and H4IIE bioassay that we first demonstrated a gradient of responses that was strongly correlated with the concentrations of TEQs (25) (Figure 2).

In addition to the relationship observed between TEQ and rates of mortality of double-crested cormorant eggs, we observed strong correlations between concentrations of TEQs and rates of deformities in both cormorant and Caspian tern chicks (51) (Figure 3). These observations provide evidence to support the hypothesis that PCDH are always associated with, if not the sole cause of, the observed symptoms in CFEWB reproducing on the Great Lakes.

Six criteria to be used in determining the validity of ascribing chemical causes to effects in ecoepidemiological studies of wildlife populations have been formalized by Fox (33). These are consistency of observations, strength of the association, specificity of the association, time sequence, coherence, and the predictive power of the relationship. On these bases, an informed judgment can be made with a great degree of certainty that PCDH have influenced populations of wildlife species (2, 3). The weight of evidence, based on laboratory and field studies, indicates that the effects currently observed in CFEWB reproducing on the Great Lakes are caused by planar dioxin-like compounds. This is particularly true because correlations of symptoms with other PCDH, such as DDE and other "hard pesticides," are poor—certainly much less strong than the correlation with TEQs.

Because ecoepidemiological studies are correlational, often no single chemical cause can be isolated in wildlife to which symptoms can be assigned. Ideally, one would apply

not only Fox's ecoepidemiological criteria (33), but also Koch's postulates to wildlife contamination problems in the search for cause-effect relationships. Koch's postulates, paraphrased to be applicable to toxicants, state that after a putative causal agent has been identified and correlated with an effect, that agent must be reintroduced into unexposed animals and cause the same effects that were correlated with the toxicant under field conditions (52).

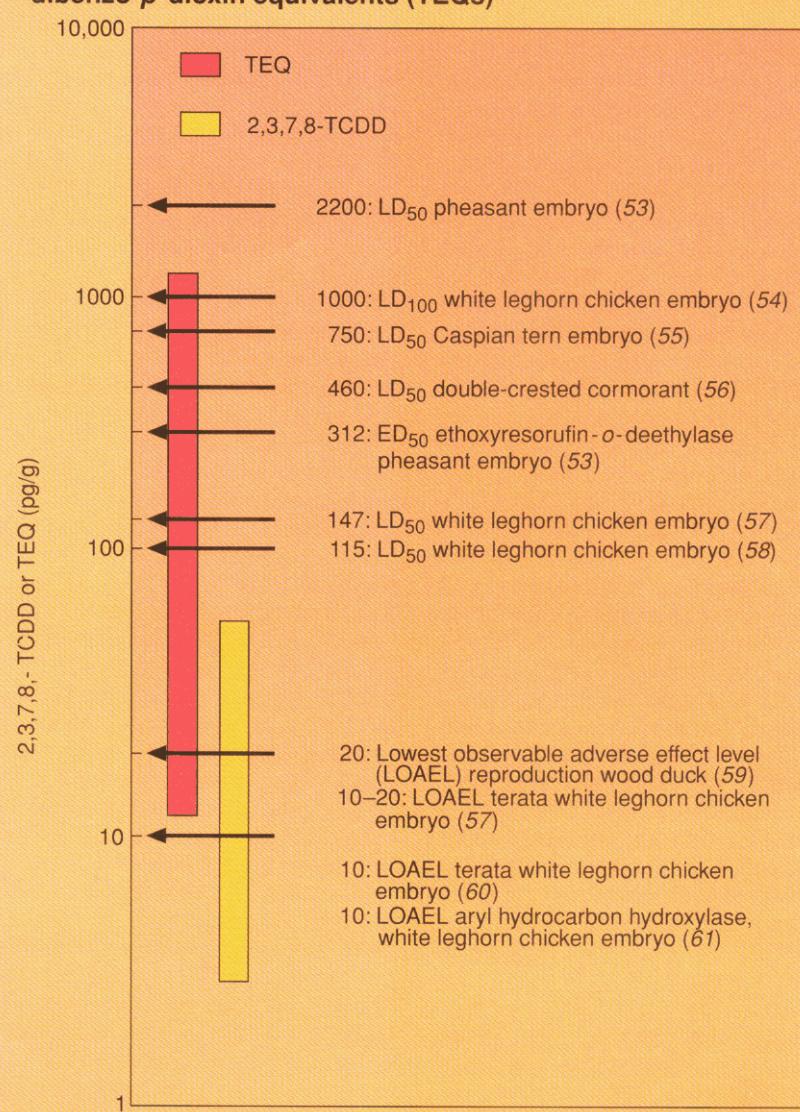
It is difficult to conduct controlled laboratory studies with wildlife species and to conduct studies with sample sizes large enough to allow sufficient statistical power to test hypotheses about effects, such as deformities. It is also difficult to conduct studies with known exposures to the same complex mixtures to which wildlife are exposed under field conditions. Regarding CFEWB on the Great Lakes, we feel we have completed the first four of Koch's postulates. It is likely that the effects observed are caused by the TEQ contributed by PCDD, PCDF, and PCBs.

Toward completion of Koch's fifth postulate, we have fed fish from the Great Lakes to chickens (unpublished information). The concentrations of PCDH were characterized instrumentally and with the H4IIE assay. The same types of symptoms observed under field conditions were induced in the surrogate species under laboratory conditions. We are currently conducting studies in which complex mixtures, subfractions, and artificial cocktails of planar PCDH are being injected into eggs of chickens and double-crested cormorants, to further elucidate which specific congeners are responsible for the observed effects.

Contributions of individual PCDH

The proportion of TEQ contributed by PCDD and PCDF congeners in environmental samples from the Great Lakes region ranges from 2% to 50% in CFEWB (24). The proportion of the TEQ contributed by the planar PCBs is equal to or greater than that contributed by PCDD and PCDF. In carp from Saginaw Bay when TEFs based on induction of EROD in H4IIE cells were used to calculate TEQs, approximately half of the TEQs were contributed by PCDDs and PCDFs. The other calculated TEQs were contributed by PCB congeners. The use of other TEF values results in different proportions of the total, as well as different absolute concentrations of TEQs. A similar relative importance

FIGURE 1
Thresholds for avian effects of 2,3,7,8-TCDD and tetrachloro-dibenzo-p-dioxin equivalents (TEQs)



The hatched and solid bars represent the relative range of concentrations (pg/g) of TEQ and 2,3,7,8-TCDD, respectively, that are currently found in the eggs of colonial fish-eating water birds of the Great Lakes. Arrows indicate various toxicity thresholds for avian species that have been tested in laboratory or field studies. Reference numbers are in parentheses.

of planar PCBs has been reported in marine mammals (62) as well as in a variety of samples from Sweden (63).

In contrast to the fish-eating birds in the Great Lakes, "terrestrial" avian species examined in Green Bay contained lesser concentrations of TEQ, and the relative proportion of the TEQ, which was contributed by PCDDs and PCDFs, was greater than that in the fish-eating species (24). PCDDs and PCDFs comprised 3.2–71% of the TEQ in the terrestrial, avian species (24). However, the absolute concentrations of TEQ contributed by PCDDs and PCDFs were similar in both types of birds. This suggests that there is a widespread background exposure of all

species to PCDD and PCDF. PCDD and PCDF were contributed as contaminants in the Aroclor 1242, but this could not account for the TEQ observed in the bird tissues.

There is an additional trophic-level transfer in CFEWB and their eggs as a result of foraging on fish. Therefore, they have a greater bioaccumulation potential than the terrestrial species. Also, there are known local sources of PCBs, whereas the sources of PCDD in Green Bay may be more distant or caused by atmospheric deposition. PCDF are readily depurated by birds and generally do not accumulate to any great extent.

Currently, much of the discussion

of the safety of people consuming fish flesh from the Great Lakes is centered on the concentration of TEQ contributed by the PCDD and PCDF. However, the overall contribution to the concentrations of TEQ from both PCDD and PCDF is generally less than 50% of the total TEQ present in a sample. Because the effects currently observed are thought to be caused by the TEQ, and their concentration is small relative to other compounds, the TEQ will be the class that will determine the level of remediation and environmental protection necessary to protect wildlife populations of the Great Lakes at this time. Because the planar PCBs make a significant contribution to the total TEQ, they should be emphasized in wildlife and human hazard and risk assessments.

Selective enrichment of PCDH

Chemical weathering and biomagnification can result in patterns or relative concentrations of PCDH congeners that are different from the technical mixtures and different from one location to another (64). These patterns can change over time (65), such that they are significantly different from those of the original technical mixtures that were released to the environment (64). Also, there can be changes in the relative pattern of accumulation in the ecosystem as trophic biomagnification occurs (66, 67).

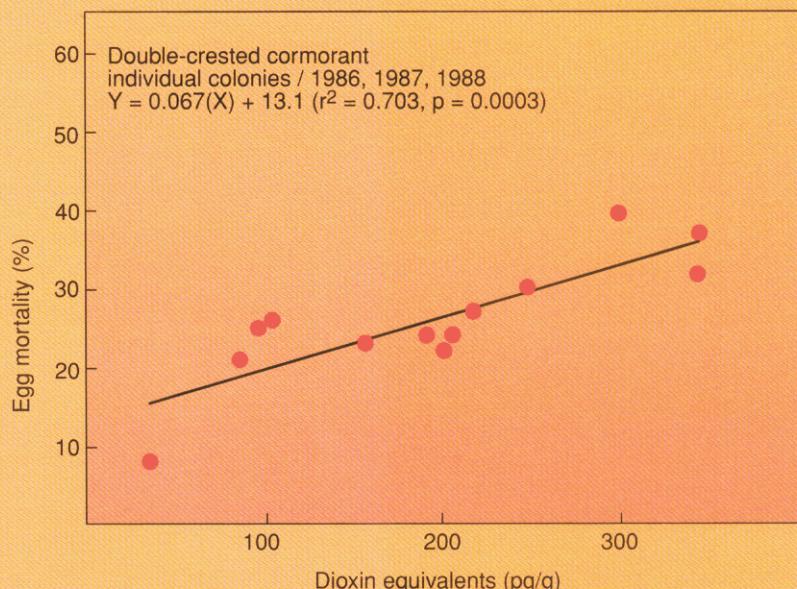
Selective accumulation of the more toxic PCB congeners can result in a mixture in the tissues of target animals that is more toxic than would be predicted from an estimate of the original Aroclor mixture. This enrichment of the more toxic, non-ortho-substituted PCB congeners results in a relative toxic potency of the mixture that is 4–6 times greater than that of the original technical mixture (24–26, 55).

The concentrations of TEQ measured in the eggs of CFEWB are greater than can be accounted for by the contribution from the Ah-r active congeners of PCBs, PCDDs, and PCDFs (26).

When the TEQ/total PCB ratio was calculated for samples from Caspian tern eggs it was found that it varied among locations and that adverse effects were more closely related to concentrations of TEQ than to the concentrations of PCBs (52, 55–56).

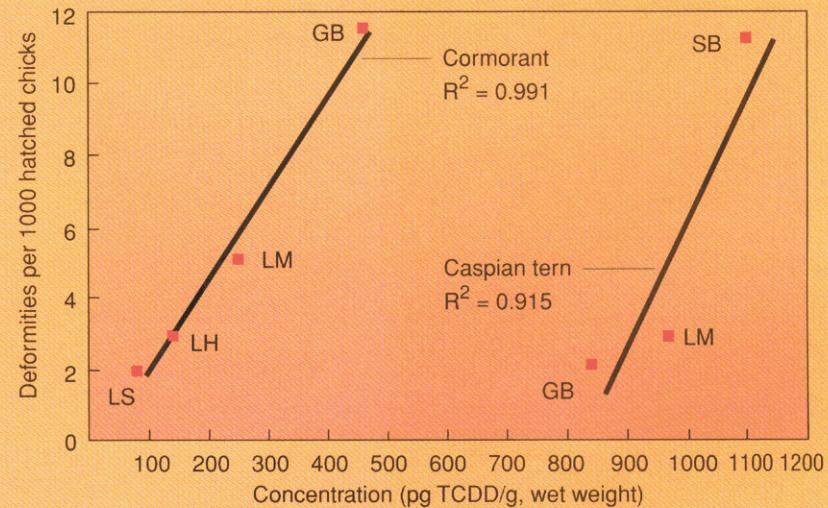
Current understanding of the mechanisms by which these complex mixtures cause biological effects does not indicate nonadditivity, but rather a greater concentration in eggs

FIGURE 2
Embryo-lethality of double-crested cormorants from 13 colonies on (and one location off) the Great Lakes as a function of TEQ



Reprinted with permission (25).

FIGURE 3
Deformities in the embryos and chicks of double-crested cormorants and Caspian terns in the Great Lakes



SB = Saginaw Bay, GB = Green Bay, LM = Lake Michigan, LH = northern Lake Huron, LS = Lake Superior. Reprinted with permission (22).

than would be attained by injecting Aroclor mixtures into eggs. Historically, total concentrations of PCBs have been compared to observed adverse effects. Although concentrations of PCBs are often correlated with adverse effects, the correlation is often poor and concentrations of TEQ are better correlated with effects, even though PCBs seem to contribute the greatest portion of the TEQ (25). The relative potencies of extracts of CFEWB eggs from Green Bay ranged from 6 to 56 pg TEQ/ μ g

PCB, which indicates that total PCB content of a sample is a poor indicator of the biological potency of the toxicity mediated through the Ah-receptor, even though the measured concentrations of TEQ were correlated with the total concentration of PCBs.

Ah-r active compounds

The three classes of planar PCDH are prevalent in the environment and seem to account for the majority of the adverse effects observed in

the CFEWB of the Great Lakes region. But in addition there are a number of chlorinated and non-chlorinated compounds that, based on either *in vitro* or *in vivo* experimental evidence, are known to cause similar adverse effects or, because of their structure, might be expected to cause effects through the Ah-receptor mediated mechanism (see box listing compounds).

When concentrations of TEQ are determined by the H4IIE bioassay or predicted from the use of H4IIE-derived TEFs in conjunction with concentrations of PCDH, there is good agreement between the two methods in small fishes, including predatory fishes. However, with larger (older) fishes or birds the TEQ predicted from PCBs, PCDDs, and PCDFs sometimes underestimates the concentrations of TEQ measured by the H4IIE bioassay (24). For instance, in large carp from Saginaw Bay, congeners from these three classes of compounds account for only 25% of the TEQ measured in the H4IIE bioassay, whereas in eggs and chicks from several species of birds from Green Bay, approximately 50% of the H4IIE bioassay-derived TEQ are accounted for by concentrations of PCBs, PCDDs, and PCDFs (24).

Some of these other potentially Ah-r-active PCDH are currently used in commerce and should receive special attention during promulgation of regulations and environmental monitoring. In addition to the listed compounds, it is possible that other halogenated or substituted analogues might also be active. Not all of these compounds have been identified in the environment, but many have been found to occur at concentrations that might be ecotoxicologically significant (22, 24, 68). Thus, we advocate the use of the H4IIE bioassay system to determine whether all of the potentially active compounds have been considered in hazard assessments and monitoring programs.

Conclusions

Current concentrations of PCDD, PCDF, and PCBs in Great Lakes piscivorous birds and their prey are less than those during the 1960s and 1970s. Some bird populations, such as double-crested cormorants and herring gulls, have made dramatic recoveries since that time. However, populations of other species, such as the common and Forster's tern, continue to decline. The concentrations of TEQ in several spe-

Compounds that may have the potential to cause adverse effects through the Ah-r mediated mechanism of action (depending on experimental evidence or structure)

Polycyclic aromatic hydrocarbons	Polychlorinated fluorenes
Polychlorinated biphenyls	Polychlorinated dihydroanthracenes
Polychlorinated dibenzo-p-dioxins	Polychlorinated diphenylmethanes
Polychlorinated dibenzofurans	Polychlorinated phenylxylyethanes
Polychlorinated naphthalenes	Polychlorinated dibenzothiophenes
Polychlorinated diphenyltoluenes	Polychlorinated quaterphenyls
Polychlorinated diphenyl ethers	Polychlorinated quaterphenyl ethers
Polychlorinated anisole	Polychlorinated biphenylenes
Polychlorinated phenoxy anisoles	Polychlorinated thioanthrenes
Polychlorinated xanthenes	Polybrominated diphenyl ethers
Polychlorinated xanthones	Polychlorinated azoanthracenes
Polychlorinated anthracenes	

cies appear to be greater than the threshold for discernable, population-level effects at several locations around the Great Lakes. For instance, subpopulations of double-crested cormorants and Caspian terns in Saginaw and Green Bays continue to display abnormally great rates of developmental deformities and embryo lethality.

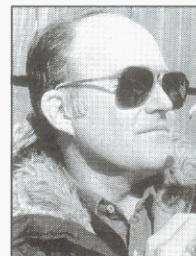
In general, all of the populations of CFEWB on the Great Lakes are displaying symptoms of exposure to chlorinated chemicals at the biochemical level. These exposures are still causing lethality and deformities in embryos of all of the populations that we have examined. The observed effects are greater than those observed in less contaminated populations off the Great Lakes; however, these effects are translated into biologically significant population-level effects only in the more contaminated areas, such as Saginaw and Green Bays.

The results of laboratory and field studies indicate that the lethality of and deformities in embryos of CFEWB of the Great Lakes are caused by the toxic effects of multiple compounds, which express their effects through a common mechanism of action. In addition, the use of TEQ values seems to explain the observed effects better than single instrumental measurements of individual compounds.

Several sets of TEF values have been proposed, the use of which results in different estimates of absolute concentrations of TEQ in fish and wildlife. Although the rank-order of relative potency of these compounds seems to be similar and will result in integrative measures, which correlate well with effects in populations, we caution against the indiscriminate use of these techniques. The results of the additive model or bioassays need to be spe-



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cies- and endpoint-specific and calibrated to the response of interest before they can be used to predict the dose-response relationships or thresholds for effects. When appropriate TEFs are applied to the concentrations of individual congeners of PCDD in tissues of CFEWB, the planar PCB congeners account for the greatest proportion of the TEQ predicted by the additive model.

We advocate the use of integrating bioassays, such as the H4IE assay or similar bioassay systems based on other cell lines, to account for the presence of compounds that may not be quantified instrumentally and to account for possible interactions among more PCDD and their antagonists. These techniques can best be used in concert with selective extraction, enrichment, and fractionation techniques interfaced with instrumental analyses to understand and reconcile observed and predicted effects of complex mixtures. When this is done for samples of fish or bird tissues from the Great Lakes, it is found that there are TEQ that cannot be accounted for by concentrations of individual congeners of PCDD, PCDF, and PCBs.

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