

## Planar Chlorinated Hydrocarbons (PCHs) in Colonial Fish-Eating Waterbird Eggs from the Great Lakes

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### ABSTRACT

*Reproductive impairment of double-crested cormorants (Phalacrocorax auritus) and Caspian terns Hydroprogne caspia) has recently been observed in the Great Lakes of North America. Planar chlorinated hydrocarbons (PCHs), which include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDS), and polychlorinated dibenzofurans (PCDFs), are believed to be at least partially responsible for the increased incidence of teratogenesis and embryonic mortality. The objective of this study was to assess the potency of the PCH mixtures in egg extracts from these colonial waterbirds. The relative potency of the egg extracts was assessed by their ability to induce cytochrome P-450-dependent ethoxyresorufin O-deethylase (EROD) in H4IIE rat hepatoma cells. The magnitude of the response was compared with EROD induction in cell cultures by a standard, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The TCDD-equivalents derived by this bioassay concur with residue analysis and biological data, in that the highest TCDD-equivalents were found in waterbird egg composites from areas with greater PCH concentrations and more severe reproductive effects. Significant concentrations of PCHs were detected in all sites tested; the range of TCDD-equivalents in the waterbird eggs was 49-415 pg/g, uncorrected for extraction efficiencies. The evidence is strong for at least a partial role of PCHs as causal agents in the reproductive impairment of fish-eating waterbirds from the Great Lakes of North America.*

Colonial fish-eating waterbirds in the Great Lakes of North America have shown reproductive, immunological and biochemical symptoms similar to

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the effects observed in laboratory studies of avian species exposed to polychlorinated hydrocarbons (PCHs).<sup>1</sup> Severe egg mortality (up to 72%) and fledgling mortality (up to 100%) has recently been observed in particular colonies of double-crested cormorants and Caspian terns in this region.<sup>2</sup> Reproductive success in these species is lower in those areas with greater degrees of PCH contamination (i.e. Saginaw Bay-Lake Huron and Green Bay-Lake Michigan). However, a biological gradient correlating PCH residues in the waterbirds or their eggs and reproductive performance (i.e. hatching success) of the waterbird colonies has not been demonstrated. The reason for this apparent lack of a quantitative correlation between PCH residues and waterbird reproduction may be due to the conventional methods of residue analysis which cannot account for complex interactions of these mixtures. The individual PCH congeners/compounds have drastically different biological potencies, which are not accounted for by quantitation against a standard mixture (i.e. Aroclor, Kanachlor or Clophens). Even if reliable determination of PCH residues is performed on an isomer/congener specific basis, the complex interactions of synergism, antagonism or additivity which are known to occur,<sup>3</sup> cannot be estimated in this fashion. Various researchers have used the ability of PCHs to induce cytochrome P-450-dependent, 3-methylcholanthrene (3-MC) type of catalytic activity in H4IIE rat hepatoma cells to assess the potency of PCH mixtures.<sup>4,5</sup> Using individual PCH congener induction potency in these cells is strongly correlated to weight loss and thymic atrophy *in vivo*.<sup>6</sup> Therefore, it was the objective of this study to use H4IIE rat hepatoma cells to assess the induction potency of PCH extracts from fish-eating waterbird eggs.

Colonial waterbird eggs were collected from 41 colonies in seven regions of the Great Lakes. These regions were: Green Bay, Lake Michigan; Saginaw Bay, Lake Huron; Thunder Bay, Lake Huron; Georgian Bay/North Channel, Lake Huron; Beaver Island, Lake Michigan; Northwestern Lake Huron; and Tahquamenon Island, Lake Superior. Samples were kept frozen at  $-20^{\circ}\text{C}$  prior to extraction. Rat hepatoma H4IIE cells (ATCC CRL 1548) were maintained under standard conditions previously described.<sup>7</sup> Waterbird eggs were extracted using techniques described elsewhere.<sup>5</sup> H4IIE cell cultures were exposed to four to five serial dilutions of the extract in iso-octane, each dilution in triplicate. After 72 h incubation, ethoxyresorufin *O*-deethylase (EROD) activity was measured in each culture.<sup>8</sup> The extract  $\text{EC}_{50}$  was compared to a TCDD standard  $\text{EC}_{50}$  for EROD induction in these cells, and TCDD-equivalents were calculated as described previously.<sup>6</sup>

Significant concentrations of TCDD-equivalents were found in colonial waterbird eggs from all regions of the Great Lakes. The range of TCDD-equivalents in the eggs was 49-415 pg/g, uncorrected for extraction

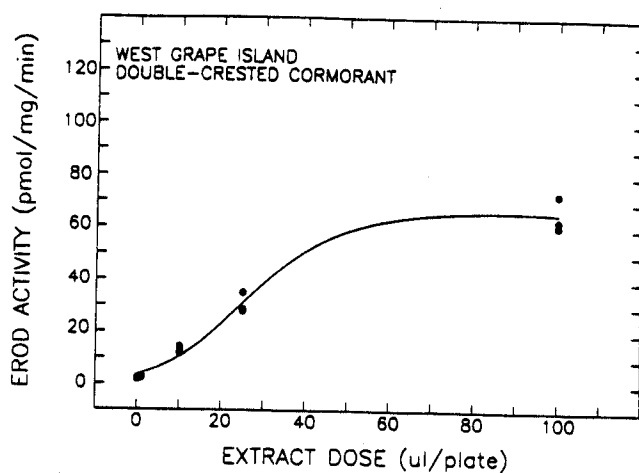


Fig. 1. Extract-H4IIE EROD induction dose-response curve. EROD = ethoxyresorufin *O*-deethylase. Colony composite waterbird egg sample (30 g) was extracted and concentrated to 1 ml final volume in isoctane. Seeding rate  $1 \times 10^6$  cells/plate 24 h prior to dose, incubate 3 days after dosage.

efficiencies. Examples of an extract and standard dose-response curves are given (Figs 1 and 2). When all colonies were ranked according to their TCDD-equivalents, the regions showed the following order of *decreasing* TCDD equivalents: Green Bay; Saginaw Bay; Thunder Bay; Georgian Bay/North Channel; Beaver Island; Northwestern Lake Huron; Tahquamenon Island. The TCDD-equivalents derived by this bioassay concur with residue

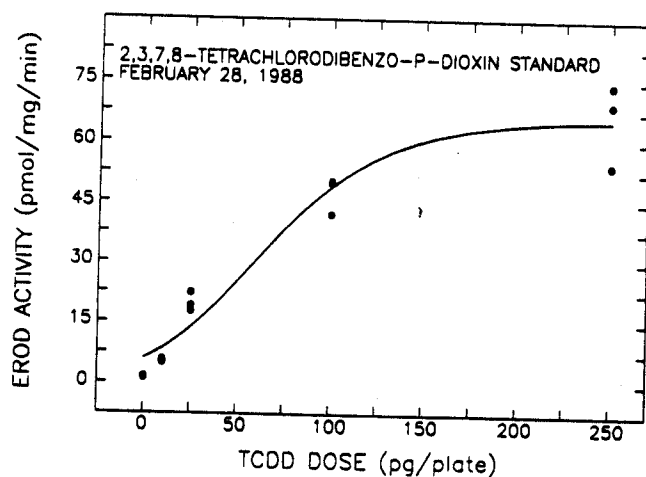


Fig. 2. TCDD-H4IIE EROD induction dose-response curve. EROD = ethoxyresorufin *O*-deethylase. TCDD delivered in 100  $\mu$ l isoctane. Seeding rate  $1 \times 10^6$  cells/plate 24 h prior to dose, incubate 3 days after dosage.

analysis and biological data from other studies,<sup>2</sup> the highest TCDD-equivalents were found in waterbird egg composites from areas with greatest PCH concentrations and most severe reproductive effects.

In conclusion, this bioassay system may be a useful tool for the assessment of complex mixtures of PCHs from environmental samples. It provides a determination of the potency of the mixture which incorporates synergistic and antagonistic interactions which can occur at the cellular level. Additionally, because the extracts are taken from the target organ, differences in pharmacokinetics among congeners and species are incorporated into this bioassay system. Further studies are required to determine whether the relative potency of PCHs in rat cells are similar to their relative potencies in other species. Until such work is complete caution must be taken when using this bioassay to assess toxic risks to other species. However, this evidence suggests at least a partial role of PCHs as causal agents in the reproductive impairment of fish-eating waterbirds from the Great Lakes of North America.

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