JA-89

ETox 2289

Reprinted from

Canadian Journal of Fisheries and Aquatic Sciences

Réimpression du

Journal canadien des sciences halieutiques et aquatiques

Bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in PCB-containing extracts from the flesh and eggs of Lake Michigan chinook salmon (*Oncorhynchus tshawytscha*) and possible implications for reproduction

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Volume 48 • Number 9 • 1991

Pages 1685–1690

Canada

Fisheries and Oceans / Pêches et Océans

Printed in Canada by The Runge Press Limited
Imprimé au Canada par The Runge Press Limited

Bioassay-Derived 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Equivalents in PCB-Containing Extracts from the Flesh and Eggs of Lake Michigan Chinook Salmon (*Oncorhynchus tshawytscha*) and Possible Implications for Reproduction

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Ankley, G. T., D. E. Tillitt, J. P. Giesy, P. D. Jones, and D. A. Verbrugge. 1991. Bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in PCB-containing extracts from the flesh and eggs of Lake Michigan chinook salmon (*Oncorhynchus tshawytscha*) and possible implications for reproduction. Can. J. Fish. Aquat. Sci. 48: 1685–1690.

Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ), derived via the H4IIE rat hepatoma cell bioassay, were measured in polychlorinated biphenyl (PCB) containing extracts of flesh (dorsal muscle) and egg samples from 10 spawning chinook salmon (*Oncorhynchus tshawytscha*) from Lake Michigan. There was a marked maternal transfer of both TCDD-EQ and PCBs, and potency of the PCB mixture (expressed as picograms of TCDD-EQ per microgram of PCB) in eggs was 2.5 times greater than potency of the PCB mixture in dorsal muscle of the fish. There was a statistically significant, inverse relationship between the total concentration of PCBs in eggs and hatching success of the fish, with an effect concentration that corresponded to approximately 100 pg TCDD-EQ/g egg. Our results, based on a relatively small sample size, suggest that PCBs, in particular those with TCDD-type activity, may have influenced reproductive success of the fish.

On a mesuré les concentrations des équivalents de 2,3,7,8-tétrachlorodibenzo-*p*-dioxine (TCDD-EQ) obtenus par bioessai cellulaire H4IIE sur les hépatomes de rat, dans des extraits de chair (muscle dorsal) et des échantillons d'oeufs de dix géniteurs de saumon quinnat (*Oncorhynchus tshawytscha*) du lac Michigan contenant des BPC biphényles polychlorés (BPC). On a observé un transfert maternel marqué à la fois des TCDD-EQ et des BPC, et l'activité du mélange de BPC (exprimé en picogrammes de TCDD-EQ par microgramme de BPC) dans les oeufs était 2,5 fois supérieure à l'activité de ce mélange dans le muscle dorsal du poisson. Il existait une relation inverse, statistiquement significative, entre la concentration totale de BPC dans les oeufs et le succès d'éclosion des poissons, avec une concentration efficace correspondant à environ 100 pg TCDD-EQ/g oeufs. Nos résultats, fondés sur un échantillon relativement petit, peuvent indiquer que les BPC, en particulier ceux dont l'activité est de type TCDD, peuvent agir sur le succès de reproduction des poissons.

Received November 6, 1990
Accepted March 15, 1991
(JA792)

Reçu le 6 novembre 1990
Accepté le 15 mars 1991

Although polychlorinated biphenyls (PCBs) have been monitored in the environment for a number of years, only recently has it been possible to reliably identify and quantify the planar congeners in the PCB mixture. Recent studies have demonstrated the presence of the planar PCBs 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl, and 3,3′,4,4′,5,5′-hexachlorobiphenyl (IUPAC Nos. 77, 126, and 169) in sediments, invertebrates, fishes, waterfowl, and aquatic mammals from a variety of freshwater and marine sites (Huckins et al. 1988; Kannan et al. 1989a, 1989b, 1989c; Kubiak et al. 1989; Niimi and and Oliver 1989a). Based on structure activity and toxicity data from mammalian studies, it has been proposed that these and other planar congeners potentially contribute the majority of the toxic potency to PCB mixtures and thus are of great toxicological significance in aquatic ecosystems (Tanabe et al. 1987; Kannan et al. 1989b; Kubiak et al. 1989).

Although it is possible to determine concentrations of planar PCBs, there still are many uncertainties as how best to assess the toxicity of mixtures that can contain more than 100 planar and nonplanar congeners which may interact with one another. Some researchers have used toxic equivalency factors (TEF), based upon potency of different congeners relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), in an additive index, to express the total toxic potential of mixtures of PCBs and related compounds such as polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) (Sawyer and Safe 1985; Eadon et al. 1986; Kannan et al. 1988; Kannan et al. 1989b; Niimi and Oliver 1989b; van Zorge et al. 1989; Bellward et al. 1990; Olafson et al. 1990). This approach is reasonable because planar PCBs and the toxic 2,3,7,8-substituted PCDFs and PCDDs (for which TCDD is the prototype compound) all appear to have the same mode of action (Neal 1985);

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however, there are important uncertainties associated with the approach. For example, because there is a limited database on the relative potency of some congeners, in many instances it is difficult to assign realistic potency values to individual PCBs. Moreover, there is no assurance that an additive model is the best predictor of toxicity of mixtures of planar and nonplanar PCBs. Dependent upon exposure conditions, studies with different combinations of PCBs, PCDFs, and PCDDs suggest that interactions among congeners may range from antagonism (Bannister et al. 1987; Biegel et al. 1989) to additivity (Sawyer and Safe 1985; Weber et al. 1985; Birnbaum et al. 1987; Pluess et al. 1988), to, perhaps, synergism (Birnbaum et al. 1985; Bannister and Safe 1987). Ideally, from a hazard assessment standpoint, a simple measure of the biological potency of complex mixtures of PCBs and/or complex mixtures of PCBs, PCDFs, and PCDDs would be extremely useful in obviating problems associated with the assumptions involved in "traditional" TCDD-TEF evaluations. Also, it might be predicted that an integrated biochemical measure of potency would be a reasonable proximate predictor of the biological effects of these types of compounds.

In the late 1970s, researchers with the U.S. Food and Drug Administration suggested that a simple bioassay, based upon measurement of the induction of cytochrome P-450-associated monooxygenase activity in the H4IIE rat hepatoma cell line, could be a useful tool in determining the presence of TCDD and similar compounds in environmental samples (Bradlaw and Casterline 1979; Bradlaw et al. 1980; Casterline et al. 1983). In subsequent studies, Safe and co-workers have demonstrated that induction of either arylhydrocarbon hydroxylase or 7-ethoxyresorufin-*O*-dethylase (EROD) activity in the H4IIE cell line by PCB, PCDF, and PCDD congeners either singly or in combination, correlates well with the in vivo toxicity of these compounds to rats (Bandiera et al. 1984; Leece et al. 1985; Mason et al. 1985, 1986; Safe et al. 1987, 1989). More recently, Zacharewski et al. (1989) and Tillitt et al. (1989, 1991a, 1991b) demonstrated the utility of the H4IIE bioassay for assessing the potency of various combinations of complex mixtures of PCBs, PCDFs, and PCDDs in samples of fishes and birds from the Great Lakes.

The objective of this study was to use the H4IIE bioassay to determine the biological potency, relative to TCDD, of the PCB mixture in extracts of flesh and egg samples from female chinook salmon (*Oncorhynchus tshawytscha*) from Lake Michigan. This particular focus was chosen because previous studies had documented adverse reproductive impacts, which were hypothesized to be due to contaminants such as PCBs, in both chinook salmon and lake trout (*Salvelinus namaycush*) from Lake Michigan (Mac 1988). In addition to collecting information concerning the potency and concentrations of PCBs in flesh and egg samples from the fish, correlations between PCB potency or concentration and reproductive success were examined.

Materials and Methods

Sample Collection and Reproduction

A detailed description of collection of the fish and evaluation of their reproductive success is given elsewhere (Ankley et al. 1989a, 1989b). Briefly, 30 female chinook salmon were collected and their eggs and flesh sampled. Triplicate subsamples of fertilized eggs from each fish were incubated and hatching success and fry survival to swim-up were determined. For the present study, samples of flesh and eggs from a subset of 10 of the fish, five exhibiting the best and five exhibiting the worst reproductive success, were selected for further evaluation.

Chlorinated organic hydrocarbons in samples of dorsal muscle and eggs were extracted by the method of Ribick et al. (1981, 1982). This consisted of initially grinding the samples with anhydrous sodium sulfate, followed by column extraction with dichloromethane, gel permeation chromatography, Florisil and silica gel clean-up steps, and, finally, for samples used in the H4IIE bioassay, solvent transfer into isooctane. This technique is quite specific for PCBs; the final fraction is free of detectable PCDFs, PCDDs, and polar pesticides and contains only a few of the organochlorine pesticides (Ribick et al. 1981, 1982). Although it was impossible to characterize extraction efficiency for the PCBs (i.e. spiked compounds could interfere with results of the bioassay), efficiencies on the order of 90–98% for the method have been reported (Ribick et al. 1981, 1982), and in our experience, efficiencies for the extraction of PCBs from fish flesh and eggs are quite similar (data not shown).

Cell Culture and Bioassay Procedure

Detailed descriptions of cell culture and enzyme assay techniques were given by Tillitt et al. (1991b). Briefly, H4IIE cells were seeded into Petri dishes (15 × 100 mm) at 0.8×10^6 cells/plate in 10 mL of culture media. After a 24-h incubation period, plates were dosed with four concentrations of each tissue extract, five concentrations of TCDD (1, 10, 25, 100, and 250 pg TCDD/plate) as a standard curve, or isooctane as a solvent blank. The amount of isooctane in all treatments was 1%, a concentration that does not elicit overt toxicity or affect enzyme induction in the cells (Tillitt et al. 1991b). Dosed cells were incubated for 72 h and were then rinsed with phosphate-buffered saline and harvested into Tris–sucrose (0.05–0.2 M) buffer. Protein in the cell suspensions was determined using the method of Lowry et al. (1951), and the cells were diluted to a protein concentration of 1 mg/mL with the Tris-sucrose buffer. EROD activity was determined by the method of Pohl and Fouts (1980). Final reaction mixtures contained (in 0.1 M HEPES buffer, pH 7.8) 5 mM glucose-6-phosphate, 5 mM $MgSO_4$, 3.5 mM NADP, 2.5 units of glucose-6-phosphate dehydrogenase, 100 μg of cellular protein, and 0.05 mL of 15 μM 7-ethoxyresorufin (in methanol) in a total volume of 1.25 mL. Incubations were conducted in a shaking water bath for 10 min at 37°C and were terminated by the addition of 2.5 mL of methanol. Resorufin in the supernatant was determined fluorimetrically (550 nm excitation, 585 nm emission) and quantified by comparison with a resorufin standard.

PCB Determination

Total concentrations of PCBs in tissue and egg extracts were determined using gas chromatography with electron capture detection (GC-ECD) and quantified using the method of Burkhard and Weininger (1987). These extracts were splits of the final silica gel fraction used for the H4IIE bioassay and were spiked with 2,4,6-trichlorobiphenyl (IUPAC No. 30) as a quantification standard prior to GC-ECD.

Statistical Analysis

Concentrations of TCDD equivalents (TCDD-EQ) in the flesh and egg extracts were determined using the slope ratio

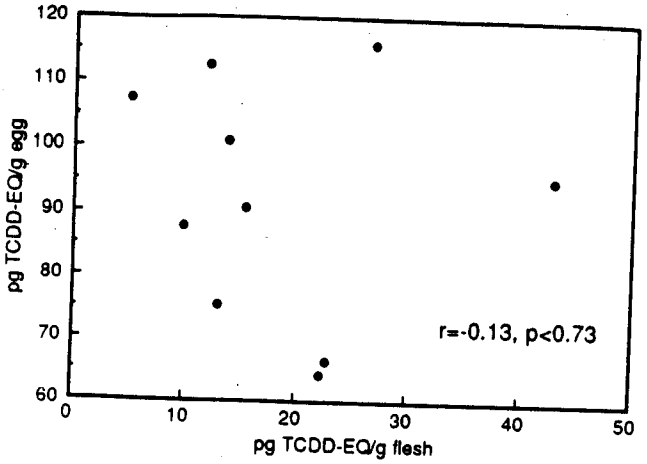

FIG. 1. Relationship of concentrations of TCDD-EQ in flesh to concentrations of TCDD-EQ in eggs from 10 Lake Michigan chinook salmon.

method of Finney (1978) by comparison with standard curves generated with TCDD. The slope-ratio method, rather than the approach described by Tillitt et al. (1991b), was used in the present study because maximal enzyme induction was not observed in cells dosed with the flesh extracts. The limit of quantification (LOQ) for the flesh and egg samples for this study was approximately 5 pg TCDD-EQ/g wet weight tissue.

Statistical manipulations, including Pearson pairwise correlations, simple linear regression, and *t*-tests, were performed using a standard computer package (Ryan et al. 1985).

Results and Discussion

TCDD-EQ were at detectable concentrations in all instances except in one flesh sample for which, although there appeared to be some enzyme induction, the slope of the dose–response curve was not significantly different from zero. In subsequent data summaries and statistical analyses, this sample was assigned a value of 2.5 pg TCDD-EQ/g (1/2 LOQ; Haas and Schett 1990).

The mean (± standard deviation) concentration of TCDD-EQ in the chinook salmon egg samples was 91.6 ± 18.4 pg/g, while the corresponding value in the flesh samples was approximately 5 times lower at 18.4 ± 11.2 pg/g. On a fish-by-fish basis, there was no apparent relationship between TCDD-EQ in the eggs and in the flesh (Fig. 1). Concentrations of TCDD-EQ in flesh of the chinook salmon were similar to those reported by Zacharewski et al. (1989), who used the H4IIE bioassay to determine the potency of extracts of chlorinated hydrocarbons from the flesh of fish from several of the Great Lakes, including Lake Michigan. Concentrations of TCDD-EQ in both egg and flesh samples from the chinook salmon were less (as much as an order of magnitude) than H4IIE-derived concentrations of TCDD-EQ in eggs of fish-eating birds such as terns and cormorants from Great Lakes sites known to be contaminated with PCBs, PCDFs, and PCDDs (Tillitt et al. 1989; 1991a).

Total PCBs were easily detectable by GC-ECD in all of the flesh and egg samples. The mean (± standard deviation) concentration of PCBs in the eggs was 3.80 ± 1.06 μg/g wet weight, while the mean concentration in samples of flesh was approximately 2.5 times lower at 1.50 ± 0.44 μg/g. Total concentrations of PCBs in both eggs and flesh were comparable with those recently reported for other collections of chinook salmon from the eastern shore of Lake Michigan (Giesy et al. 1986; Williams et al. 1989). As was true for concentrations of TCDD-EQ, there was no definable relationship, on a fish-by-fish basis, between concentrations of PCBs in eggs and flesh of the female salmon (data not shown).

There were strong, statistically significant linear relationships between concentrations of TCDD-EQ and PCBs in both the egg and dorsal muscle samples (Fig. 2). This provides evidence for the dominant contribution of PCBs to the concentrations of TCDD-EQ in these chinook salmon extracts.

The fact that TCDD-EQ measured in the eggs were 5 times greater than TCDD-EQ in the dorsal muscle, while total PCBs were only 2.5 times greater, is interesting. This suggests that if PCBs were the only compounds in the samples determining TCDD-EQ, the PCB mixture in the eggs was enriched relative to that in the flesh. To quantify this observation, the concentration of TCDD-EQ was divided by the concentration of PCBs in each sample. Thus, potency of the mixture is expressed as TCDD-EQ per unit PCBs. On this basis, the flesh samples had a mean (± standard deviation) potency of 11.80 ± 4.23 pg TCDD-EQ/μg PCB, while the egg samples were significantly greater ($t = 6.7$, $p < 0.0001$) at 24.75 ± 4.42 pg TCDD-EQ/μg PCB. Again, assuming that PCBs were the primary contributors to TCDD-EQ in the samples, this indicates that potency of the PCB mixture was about 2.5 times greater in eggs

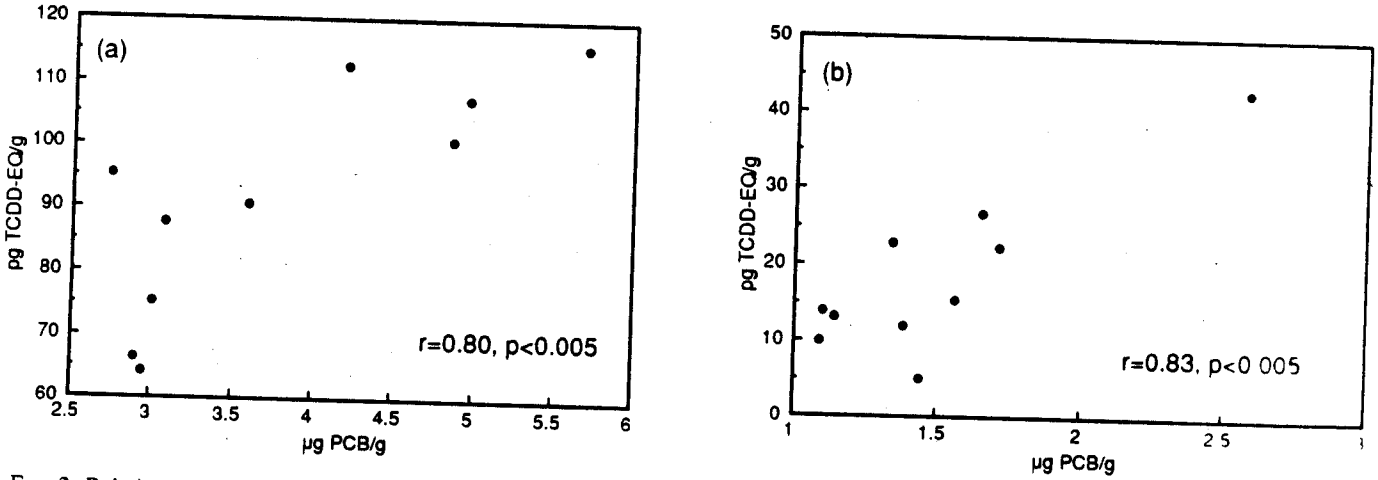

FIG. 2. Relationship between concentrations of TCDD-EQ versus PCBs in (a) eggs and (b) flesh from 10 Lake Michigan chinook salmon

TABLE 1. Correlations of concentrations of TCDD-EQ and PCBs in eggs and flesh with hatching success and fry survival for chinook salmon from Lake Michigan.

Contaminant measure	Reproductive parameter	
	Hatch	Fry survival
TCDD-EQ (flesh)	0.46 (0.18)[a]	0.26 (0.50)
TCDD-EQ (eggs)	−0.51 (0.13)	−0.37 (0.33)
PCBs (flesh)	0.17 (0.64)	0.34 (0.37)
PCBs (eggs)	−0.75 (0.01)	−0.57 (0.11)

[a] r(prob > r).

as compared with dorsal muscle. Recently, others have found that enrichment of PCB potency (determined via the H4IIE bioassay; Tillitt et al. 1991a), as well as concentrations of planar PCB congeners (Kannan et al. 1989a), relative to original PCB mixtures (e.g. Aroclors), can occur in biota. However, this is the first observation that differences in PCB potency could occur among different tissues within an organism. An extremely important implication of any sort of enrichment of PCB potency is, of course, that hazard assessments based upon total PCBs will be inaccurate.

There are at least two possible explanations for the observation of enhanced PCB potency upon maternal transfer. It may be that concentrations of planar PCB congeners were greater in eggs as compared with flesh. Alternatively, patterns of planar and nonplanar PCBs in eggs versus flesh may have been altered so as to change toxicological interactions among the congeners. For example, some nonplanar PCB congeners may act as antagonists to planar PCBs (e.g. Biegel et al. 1989); thus, if the eggs contained smaller concentrations of antagonistic congener(s) than the flesh, even with similar concentrations of the planars, the egg mixture may nonetheless have appeared more potent. More extensive chemical analyses than conducted in this study would be required to further elucidate either of these or other possible explanations for the observed differences in apparent potency of PCBs in eggs versus flesh.

Among eight correlations of the concentrations of TCDD-EQ or PCBs in eggs or flesh with two measures of reproductive success in the fish (Table 1), the only significant one was an inverse relationship between hatching success and concentrations of PCBs in eggs (Fig. 3a). The relationship between percent hatch and concentrations of TCDD-EQ in the eggs (Fig. 3b) was not significant but was interesting in that there appeared to be a threshold, with hatching success consistently reduced at egg TCDD-EQ concentrations greater than 100 pg/g. This observation may be of particular significance in light of recent findings by researchers from the University of Wisconsin, who exposed lake trout eggs to TCDD in the water. In their studies, it was found that the LD50 concentration (in eggs) for fry mortality was approximately 65 pg TCDD/g (R. Peterson, pers. comm.), a value reasonably comparable with TCDD-EQ concentrations associated with adverse effects in the chinook salmon eggs from our study.

Due to uncertainties concerning the exact composition of the PCB mixture in the eggs of the chinook salmon, it is difficult to compare our data with the results of other studies evaluating relationships between PCBs and reproductive success in fishes (e.g. Hansen et al. 1973; Nebeker et al. 1974; Hogan and Brauhn 1975; Broyles and Noveck 1979; Willford et al. 1981; Mac 1988). It is worth noting, however, that unlike our observations, Stauffer (1979) found no relationship between total PCB concentration in eggs and reproductive success in chinook salmon from Lake Michigan, but again, the composition of the PCB mixture (as well as other contaminants) present in fish from the mid-1970s probably was quite different than the present array of chemicals in Lake Michigan chinook salmon.

Our results suggest that PCBs, in particular the TCDD-related activity of PCBs, may have been related to observed adverse reproductive impacts in these Lake Michigan chinook salmon; however, our sample size of 10 is far too small to justify using the present study as conclusive evidence of this possibility on a lake-wide basis. Moreover, it is impossible to eliminate possible effects of other contaminants likely present in the fish. Further studies are required to corroborate (or refute) these results. For example, it would be desirable to examine reproductive success versus PCB and TCDD-EQ concentrations in chinook salmon from other locations in Lake Michigan to develop a wider dose–response range. Also, further laboratory studies with planar PCBs, PCDFs, and PCDDs (including TCDD) should be performed to provide a database for assessing TCDD-EQ (or TCDD-TEF) concentrations in terms of toxicity to fishes. Finally, it is important to focus research efforts upon documenting and explaining the apparent phenomenon of selective enrichment of PCB potency between different environmental and biological compartments. The H4IIE bioassay should prove to be an excellent tool for these types of studies.

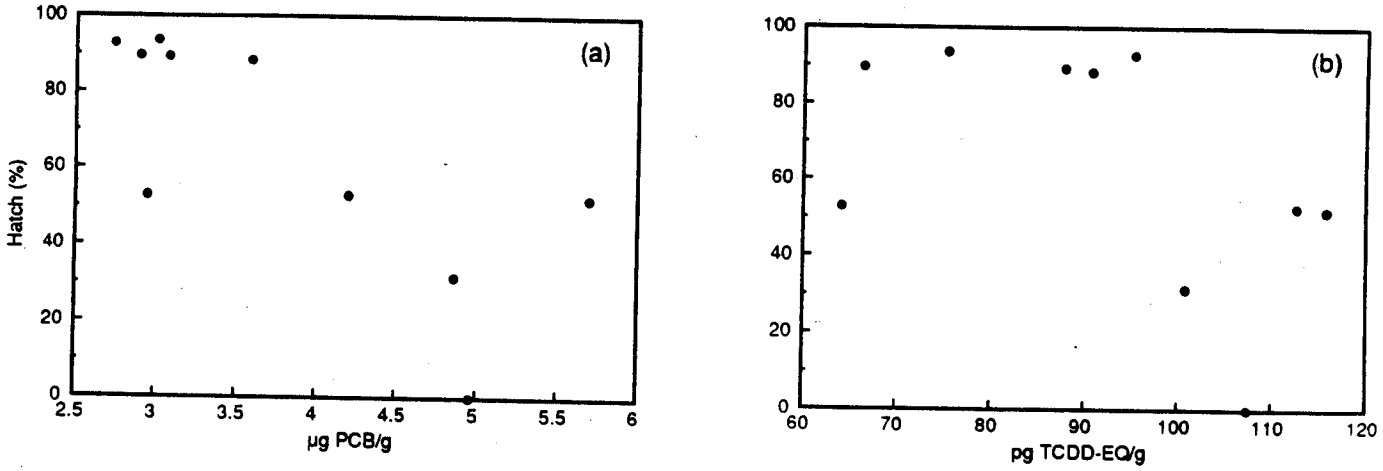

FIG. 3. Relationship of hatching success to concentration of (a) total PCBs and (b) TCDD-EQ in eggs from 10 Lake Michigan chinook salmon.

Acknowledgements

We thank all the persons who have contributed to this study. They include D. Ankley, R. Hoke, L. Williams, J. Newsted, C. Rosiu, and K. Obermeyer. We also thank the Michigan Department of Natural Resources for their cooperation in collection of the fish. Dr. P. Cook provided helpful comments on an earlier draft of the paper. D. Williams typed the manuscript and R. LePage prepared the figures. Support for portions of this work came from the Center for Environmental Toxicology at Michigan State University. This work was also supported with funds from grant No. NA 89AA-D-SG083 from the National Sea Grant College Program, National Oceanographic and Atmospheric Administration, U.S. Department of Commerce, and funds from the State of Michigan.

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