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Environmental Contamination and Toxicology

Egg Mortality of Lake Geneva Charr (*Salvelinus alpinus L.*) Contaminated by PCB and DDT Derivatives

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Organochlorine compounds such as PCB and ΣDDT (pp'DDT + pp'DDE + pp'DDD) show a high chronic toxicity for various aquatic species (Mayer et al. 1977; Wassermann et al. 1979), particularly with respect to reproduction (Bengtsson 1980; Freeman and Idler 1975).

In earlier publication we noted a rather high contamination level of various fish species in Lake Geneva due to PCB (Monod and Keck 1982). Other studies have shown that Lake Geneva is widely contaminated by organochlorine residues (Burgermeister et al. 1983; Mowrer et al. 1982). We therefore began a field study in order to determine the potential risks to reproduction by evaluating the correlation between PCB and ΣDDT levels in the eggs of charr and their mortality rates following artificial fertilization. Indeed, many laboratory experiments stress the impact of PCB on fish reproduction. However, data obtained from field studies are rather wanting; among these studies, those of Johansson et al. (1970) indicate a relationship between PCB levels and the mortality rate of coho salmon eggs.

Charr (*Salvelinus alpinus L.*) are currently reproducing in Lake Geneva, but they are known to be highly sensitive to variations in the environment (Balon 1980; Hartmann 1983) and previous results have shown that this species is one of the most contaminated in this lake (Monod and Keck 1982).

MATERIALS AND METHODS

Wild charrs were captured on the 15th, 22nd, 16th of December 1981. The ova of 18 females were gathered individually, then fertilized with the sperm of males captured at the same moment (3 males per female). Each female was isolated and its age was determined by scalimetry). Eggs were maintained in a lake water sample (temperature ≈ 6°C) until their arrival at the fish farm 1 hour later, then each sample of eggs (a sample corresponds to one female) was divided into two parts.

The first part was incubated at a mean temperature level of 6.3 ± 0.8°C with oxygen level greater than 10 mg/L. The eggs were arranged in a single layer, varying in number from 385 to 817 per incu-

Table 1. Mortality rates and levels of PCB and ΣDDT in eggs of 18 female charr.

Female Age (years)	Total mortality (%)	Phases 1 (%)	2 (%)	3 (%)	4 (%)	P C B ppm/w.w.	P C B ppm/l.w.	Σ D D T ppm/w.w.	Σ D D T ppm/l.w.
4	5.1	1.5	0.0	3.1	0.5	0.16	16	0.10	10
4	5.7	0.0	0.0	4.3	1.4	0.14	18	0.071	8.9
4	6.7	0.2	1.1	2.4	2.9	0.17	17	0.084	8.4
4	8.3	3.2	0.0	3.2	1.9	0.12	13	0.072	8.0
4	11	0.4	3.7	5.6	0.9	0.16	16	0.076	7.6
4	13	4.0	1.5	4.5	2.6	0.22	37	0.14	24
4	25	10	12	0.5	2.3	0.28	35	0.17	21
4	25	12	8.5	2.6	2.1	0.23	38	0.11	19
3	26	10	10	1.9	3.4	0.10	10	0.036	3.6
4	28	5.3	19	1.3	1.2	0.13	16	0.045	5.0
4	29	8.3	17	1.0	2.5	0.28	35	0.13	17
3	41	20	1.8	7.9	11	0.17	34	0.045	9.0
4	50	13	35	1.0	1.0	0.14	16	0.081	9.0
5	53	7.0	36	5.9	4.1	0.50	71	0.16	23
4	59	8.0	44	2.4	5.1	0.19	27	0.096	14
4	71	17	50	1.9	2.9	0.31	44	0.12	17
3	72	21	46	1.1	3.6	0.18	45	0.096	24
6	76	35	38	0.0	2.5	0.31	78	0.17	44

Phase 1 : eggs without developed embryos (no visible cellular division); phase 2 : eggs with young embryos (from the first segmentations to blastula stage); phase 3 : eggs with aged embryos (from gastrula up to hatching); phase 4 : sac fry (from hatching up to yolk sac resorption).
w.w. = wet weight; l.w. = lipid weight

bator due to differences in egg size between spawns. The dead eggs were removed daily and preserved in a simplified Stockard solution (acid acetic : 5 vol., 40 % formalin : 5 vol., glycerin : 5 vol. and water : 85 vol.) which made possible to observe the embryos which appeared white. After hatching the dead were also counted until the resorption of the yolk sac (about 3 months after fertilization).

For each spawn the second part was analyzed for PCB and Σ DDT levels. The analytical method for extraction and purification has been described previously (Monod and Keck 1982). Analysis of ΣDDT was carried out by comparison of chromatograms obtained from crude purified extract (PCB + Σ DDT) and from the final extract (PCB + Dichlorobenzophenone).

Correlation coefficients between concentration of organochlorine residues and mortality rates were calculated (Schwartz 1963). Percentages were expressed in arc $\sin\sqrt{\text{percentage}/100}$ for variance stabilization (Lison 1958).

RESULTS AND DISCUSSION

The total mortality (from fertilization up to yolk sac resorption) was highly variable (from 5 to 76 %) between spawns (Table 1). The age of the females would not appear to be responsible for these variations. This appears clearly in the most highly represented age group (4 years), in which the mortality rates vary between 5 and 71 %. The differences in total mortality from one spawn to another correspond mainly to variations in the levels of eggs with no visible cellular division and of eggs with young embryos.

The variability observed for the mortality levels was also found with respect to concentrations in PCB and Σ DDT (Table 1). Among those compounds of the DDT group, pp'DDE was by far the most prevalent. This compound represents 2/3 of the total concentration, while the remainder was shared by pp'DDT and pp'DDD. There is a significant and positive correlation between PCB concentrations and those of Σ DDT.

Statistical evaluations were established using the combined data as illustrated in Table 1. Our results show a significant and positive correlation between PCB levels in eggs and total mortality rates providing concentrations are expressed on a lipid weight basis ($P < 0.01$). No correlation exists, however, should these levels be expressed on a wetweight basis. The correlation is equally significant between the PCB level (expressed on a lipid weight basis) and the percentage of eggs without embryos (Phase 1) ($P < 0.01$), as well as the PCB level and the percentage of eggs with young non-surviving embryos (Phase 2) ($0.01 < P < 0.02$). On the other hand, no correlation appears between the contamination of the eggs and the percentage of observed mortality during the last phases of the development (phases 3 and 4). Similar results are obtained if one considers the contamination by Σ DDT.

Despite the possibility of errors resulting from the manipulation of the gametes, our results clearly indicate that the mortality level observed from the fertilization of ova to yolk sac resorption increases proportionaly with respect to increase in the contamination level. However, this is the case only when the concentrations are expressed on a lipid weight basis. It seems logical, from a toxicological point of view, to express PCB and Σ DDT concentrations on the basis of simultaneously extractable lipids. The residue concentrations in the lipid reserves and in the other structures of the eggs (particularly the embryo) probably result from a dynamic equilibrium and so, the greatest toxicological pressure exists for eggs which have the least amount of lipids per unit weight. Thus, the high lipidic reserves may be considered to protect the embryo from organochlorine residues.

The toxicity of organochlorine residues should be considered from two viewpoints, the fertility of reproducers and the generation of the early stages of embryonic development.

The correlation between the level of residues and that of eggs with no visible cellular division suggests that the female fertility rate of Lake Geneva charr is influenced by organochlorine pollutants (the percentage of embryos of a given female is used to define fertility). The influence of the relative fertilization capacity of sperm may be neglected, because the ova of each female were fertilized by the sperm of at least 3 males. To our knowledge, no studies have been carried out with respect to the influence of organochlorine residues on fertility of female fish defined as the percentage of embryos. This should not be confounded with hatchability of eggs and with the fecundity of a female which is the number of eggs per unit-weight of reproducer. PCB and Σ DDT are potent enzyme inducers (Elcombe et al. 1979; Wassermann et al. 1979) and thus they can cause an increased hepatic catabolism of steroid hormones (Freeman et al. 1975; Sivarajah et al. 1978). It is therefore highly possible that the female fertility rate of lake charr is decreased due to hormonal disturbances which interfere with the development of ova (Koivusaari et al. 1984). This hypothesis becomes more substantiated if we bear in mind the close correlation between organochlorine levels in eggs and those in reproducers, providing the concentrations are expressed on a lipid weight basis (Matthews and Dedrick 1984; Monod and Keck 1982; Niimi 1983). In the present study such a correlation between organochlorine concentrations in eggs and those in corresponding female livers was observed : PCB concentrations (lipid weight basis) $P < 0.001$; Σ DDT concentrations (lipid weight basis) $P < 0.02$.

The existing correlation between the level of residues and the percentage of dead young embryos would suggest that PCB and Σ DDT have a toxic effect on the early stages of embryonic development. The death rates observed during the various later developmental stages are relatively low and not linked to organochlorine levels. The results observed in charr are similar in all aspects to those

observed by Johansson et al. (1970) in coho salmon (*Oncorhynchus Kisutch*) eggs : the effects of PCB levels which vary from 7.7 to 34 ppm (on a lipid weight basis) correlate with a mortality rate of 29 to 100 % which largely corresponds to unfertilized eggs and non-surviving young embryos. Nevertheless, some workers have noticed that yolk sac resorption is a critical phase (Burdick et al. 1964; Macek 1968; Broyles and Noveck 1979). The sensitivity of fry during yolk sac resorption would not be at all surprising, bearing in mind that this phase is characterized by consumption of the greater part of the lipid reserves. One might suppose that the organochlorine residues stocked up to that time are then liberated, thus increasing toxicity. We do not observe such a phenomenon in our experiment.

We must remember that the correlation test employed is a statistical device which allows us to measure the intensity of the binding between two variables; and so a significant result here does not necessarily imply a cause-effect relation between these variables. It is highly probable that, in this study of charr eggs in the field, PCB and Σ DDT are not the only pollutants present, and other types of pollution such as those by heavy metals (mercury or cadmium in the case of Lake Geneva) as well as pesticides and polychlorodibenzofurans should be taken into account. Furthermore the levels of toxicity we encountered, like those measured by Johansson are lower than those shown to be hazardous to reproduction by laboratory experiments in fish culture (Bengtsson 1980; Freeman and Idler 1975). This would indicate that the concentrations which would have an impact are clearly lower in those eggs derived from wild females than those contaminated in the more controlled environment of the laboratory. Such an observation would tend to substantiate the hypothesis of additive (and eventually synergistic) effects between different pollutants in the natural environment. Therefore it would be more prudent to consider PCB and Σ DDT not as directly responsible for the observed mortality but as indicators of contamination by various pollutants hazardous for charr reproduction in Lake Geneva.

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