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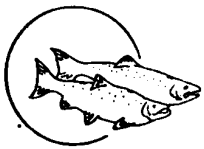

MONS 063835

PCB - Indications of effects on salmon

by

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Information concerning the effect of PCB residues in fish, or the possible hazard posed by such residues to fish, is presently lacking in the literature. Studies of the effect of the chemical on salmon and salmon reproduction has recently started at the Salmon Research Institute.

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Effects on the liver in young salmon.

"Pale liver" is a frequently appearing pathological condition among young salmon in Swedish salmon rearing stations. The condition is related to a high degree of stainable fat in the organ, as revealed by the Sudan Black B technique (JOHANSSON, 1965). There are two commonly accepted theories about the cause of this type of "fatty livers": one is based on the probably existence of a toxic substance in the organism, the other postulates a degenerative action of imbalanced diets on hepatocytes.

MILLER (1944) exposed guinea pigs, rats and rabbits to chlorinated diphenyl by injection, applications to the skin and by feeding. Liver damage - fatty degeneration, atrophy of the centrolobular cells and perinuclear basophilic granulation were present in the test groups. Consequently the primary objectives of our investigations were to study the accumulation of PCB in salmon and whether chronic exposure to sublethal levels of the chemical has a direct pathological effect on the liver.

A group of 80 one-year old salmon (average weight 18 g), raised at the institute, were used for the first test. Forty of these were exposed to a constant flow of a commercial PCB product (average 5 Cl per molecule) for two month in a concentration of 1 ppb. The rest of the fish served as controls. Both groups of fish were held in square tanks made of fiber glass (bottom area 0.9 m^2). The water flow was 10 lit/min and the water temperature 10° C $\pm$ 1. Samples were taken before the experiment began, and after 8, 12, 15, 18 and 50 days of exposure and were sent to the University of Stockholm (JENSEN) for residue analyses. At the same time samples were taken for histological examination. Livers were fixed in Bouin's Fixative mod. Hollande and 10 % Neutral Buffered Formalin pH 5.7. No mortality occured during exposure of fish to PCB.

Residue analyses showed, that the concentrations of PCB in fish from the test group and from the control group were of about the same size. (Before the experiment began: 21.1 ppm in liver on fat basis. After 50 days of exposure: test group average 60.4 ppm, controls average 42.2 ppm in liver on fat basis).

The histological examination of the material is not finished in all details, but hepatic fat occurred in practically all fishes in the test group and in the controls as well. However, in the controls the condition was possibly more slight or moderate in degree compared with the test group. Degenerating liver cells, some of them with vacuolated nuclei appeared more frequently in the test group than in the controls. Acid-fast pigment of ceroid type was noted for some fishes.

The concentrations of PCB residues in pretreatment samples and in the control group threw suspicion on occurrence of PCB-pollution of the aquatic environment. Later analyses of fish from the institute have confirmed this. To start new experiments it was necessary to get fish without basic levels of PCB-residues. After some efforts this have been done and a second test could start a few weeks ago.

Effects on salmon reproduction.

The young salmon reared at the Salmon Research Institute originates from ova taken from females grown up in the sea as well as from females reared up to maturity in fresh water at the institute. The latter females used for spawning usually have been kept in fresh water for three to eight years.

Since 1965 heavy losses of eggs originating from the females kept in fresh water have been noted, while eggs from females grown up in the sea have shown normal survival (Fig. 2A). All kinds of eggs have been incubated and handled in the same manner.

Eggs and fry have been examined for known diseases every year since 1966, but, with the exception of slight outbreaks of yolk-sac strangulation in some lots of fry in the years 1967 and 1968, we have never been able to make any true diagnosis. As most of the ova lost are supposed to be unfertilized, examinations of the gonads of parent males and females have been done. In the male gonads no structural abnormalities were found by studying stained sections in light microscope. The motility of sperm cells as well as the volume of the milt was normal. In the females, the maturing of the ovaries, expressed in egg diameter, egg number and weight of the ovary in percent of the total weight, seems to be normal compared with females grown up in the sea (JOHANSSON, to be published).

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In view of the differences in viability of ..a between the two types of parent females, and in the light of the fact that PCB pollution of the aquatic environment had been noticed, a study of the effect of PCB on reproduction in salmon was of immediate interest.

Primary it was necessary to find out whether high losses of eggs from certain females could be related to a high level of PCB residue. Nine females of different ages, (8+:1,[1] 7+:2, 6+:1, 5+:2, 4+:1 and 3+:2) and reared up to maturity in fresh water were selected for this study. Two females (ages 2.4+[2] and 2.3+), grown up in the sea served as controls (See table 1). One of these (W. 156/69) was from the river Lule älv, the other (W.D.) from the river Dalälven.

Eggs were stripped from the females on October 28 to November 10, and fertilized by milt from hatchery reared males. After water hardening, the eggs were incubated at the institute. The number of eggs in each lot varied between 2000 and 4000. The eggportion from female number 156/69 was divided into two equal lots. The one was incubated in the Heden station at the river Lule älv, the other one at the Salmon Research Institute at the river Dalälven.

Samples of eggs were taken two days after stripping and were sent to the Natl. Environment Protection Board-Special Laboratory, Uppsala for residue analyses. In some cases (see table 1) samples also were taken from eyed eggs.

The results of residue analyses are given in table 1. As the proportion of lipids present in salmonids and salmonid eggs is very variable, (the range for Salmon beeing 0.35-14 %) the concentrations are based on extractable fat.

a. Eggs from females grown up in the sea: PCB residues of 7.7 and 9.2 were noted for the samples taken two days after stripping. For female number 156/69 the concentration increased from 7.7 ppm to 14.0 ppm (6.3 ppm) during the period November to February in the lot of eggs which had been incubated at the river Lule älv, while the corresponding increase for the lot incubated at the river Dalälven were 7.7 to 19.8 ppm(12.2 ppm). The amount of fat in the ova was about the same in both lots.

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1) 8+ indicates eight years and one summer in fresh water.

2) 2.4+ indicates two years in fresh water, four years in the sea and one summer.

b. Eggs from females reared in fresh water: The PCB residues found varied from 14.4 to 34.0 ppm when analyzed two days after stripping. Thus, the PCB residues were higher in eggs from females reared in fresh water than those in eggs from females grown up in the sea. On the other hand higher concentrations of DDT were noted for the "wild" females. The total DDT values in eggs from females number 61-13, 63-01 and 65-02 are mainly due to DDE.

As shown in fig. 1, the losses of ova during incubation varies from 13 to 100 % and are smallest in the lots from "wild" females. High losses correspond to high concentrations of PCB residues. The main losses are due to unfertilized eggs or to mortalities in an early stage of development. There are no extraordinary mortalities to be found in the yolk-sac stage.

In spite of the small material used, this preliminary study indicates a possible relationship between PCB in salmon eggs and egg loss. In order to find out if this relation is true, new experiments have been prepared for the season 1970/71. For these tests females exposed to known concentrations of PCB will be used for stripping of eggs.

Since there are pronounced variations in the viability of ova and fry in different Swedish salmon rearing stations and since increased losses of eggs have been noted for some stations in the last years, also a survey of the contamination of our east coast rivers would be of great value.

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Female from family number	Female age	Organochlorine compounds in ova								% fat		% mortality
		Σ DDT fat basis		DDT fat basis		PCB fat basis		PCB fresh w. basis				
		1	2	1	2	1	2	1	2	1	2	
62-77	7+.	n.d.	n.a.	n.d.	n.a.	34.0	n.a.	1.90	n.a.	5.4	n.a.	100
65-310	4+.	n.d.	n.d.	n.d.	n.d.	27.6	30.0	1.9	1.6	6.9	5.3	99
64-10	5+.	n.d.	n.d.	n.d.	n.d.	20.2	25.0	1.2	1.0	5.9	4.2	98
63-17	6+.	n.d.	n.d.	n.d.	n.d.	26.9	34.0	1.6	1.9	5.9	5.5	82
61-52	8+.	n.d.	n.a.	n.d.	n.a.	31.7	n.a.	1.7	n.a.	5.4	n.a.	70
61-13	7+.	7.2	n.a.	n.d.	n.a.	17.5	n.a.	1.0	n.a.	5.7	n.a.	70
66-302	3+.	n.d.	n.d.	n.d.	n.d.	14.4	23.0	0.6	0.9	4.4	3.8	53
63-01	5+.	6.2	n.a.	n.d.	n.a.	15.8	n.a.	0.9	n.a.	5.8	n.a.	48
65-02	3+.	5.7	n.a.	n.d.	n.a.	15.2	n.a.	0.6	n.a.	4.2	n.a.	46
W. 156/69	2.4+	12.5	11.3	9.5	2.8	7.7	19.8	0.6	1.2	7.9	5.9	34
W. 156/69	2.4+	12.5	12.2	9.5	4.5	7.7	14.0	0.6	0.8	7.9	5.7	29
W.	2.3+	33.5	n.a.	11.6	n.a.	9.2	n.a.	0.4	n.a.	3.8	n.a.	16

Table 1. Concentrations of organchlorine compounds (ppm) in salmon ova and mortalities of eggs and sac fry from fertilization to the feeding stage; n.d., not detectable; n.a., not analysed; 1 = analysed two days after stripping; 2 = analysed from eyed eggs; W = wild female.

Σ DDT = DDT + 1.11 x (DDD + DDE).

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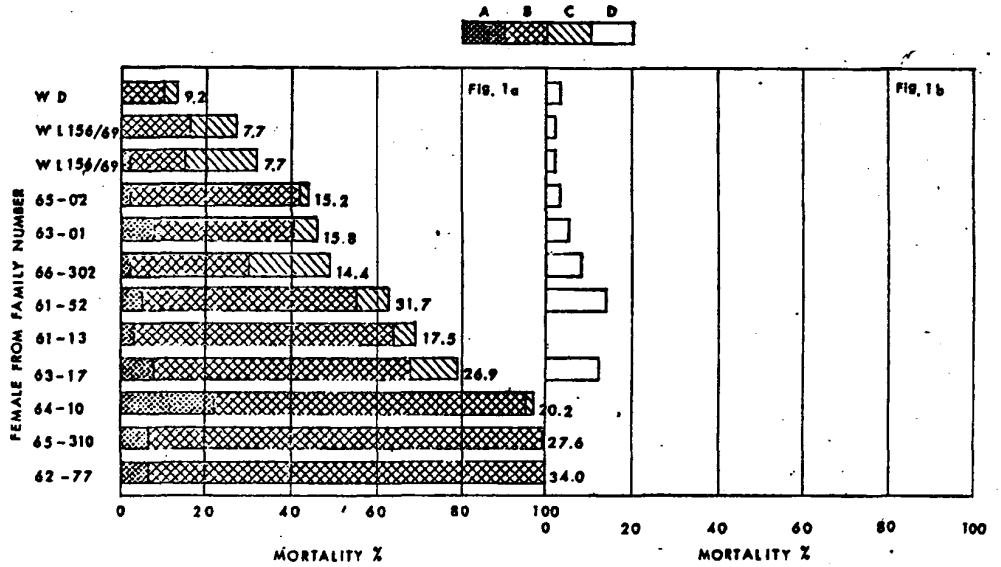

Fig. 1. Percentage of mortality during incubation (fig. 1 a) and in the yolk-sac stage (fig. 1 b); ova dead at the beginning of incubation (A); dead ova without developed embryos (B); dead ova with developed embryos (C); dead sac fry (D). W L and W D indicates females grown up in the sea. To the right of each bar is recorded the concentration of PCB residue in ova two days after stripping (ppm in fat).

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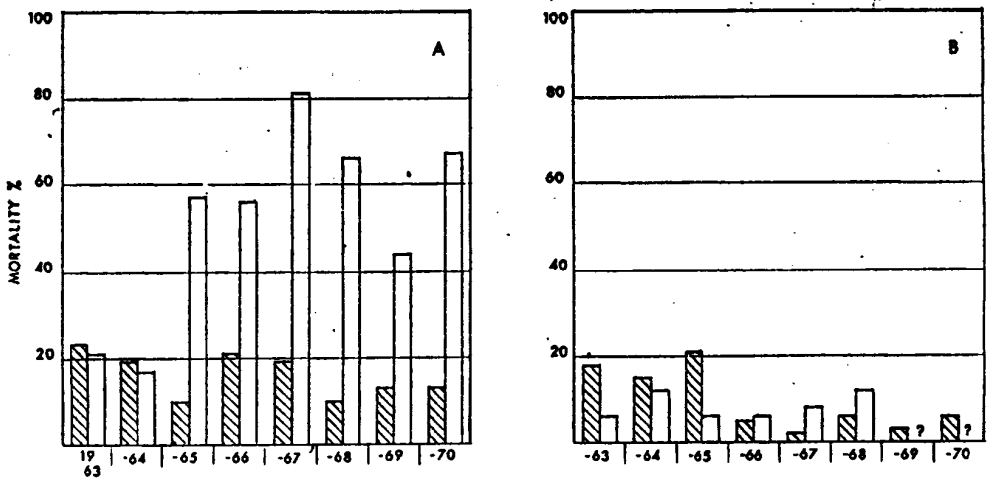

Fig. 2. Average losses of ova from fertilization to hatching (A.) and mortalities during the yolk-sac stage (B.) 1963 - 1970. Open bars = eggs and fry from females in fresh water; shaded bars = eggs and fry from females grown up in the sea.

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