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# EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON EARLY LIFE STAGES OF THE PIKE (*Esox lucius* L.)

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## ABSTRACT

Freshly fertilized pike eggs were exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at concentrations of 0.1, 1.0 and 10 ppt (ng/liter) for 96 hours. At all concentrations examined egg development was retarded by 23%, and the growth of fry was also significantly retarded for a long period after exposure. A dose-related mortality was observed. Highest mortality rates occurred during resorption of the yolk and reached almost 100 percent at a concentration of 10 ppt. Death was preceded by development of severe generalized edemas.

Histopathologically edemas and hemorrhages were observed, together with alterations of bloodvessel walls. In the liver, two stages of pathological changes were distinguished. The first was characterized by a dilation of sinusoids and a slight swelling of hepatocyte nuclei; in the second stage the nuclei were enlarged up to twice the normal diameter. Hepatocytes were degenerated and varied in size and shape and liver architecture was a almost completely lost.

## INTRODUCTION

From industrial sources 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) may occur as an environmental contaminant, particularly when 2,4,5-trichlorophenol is used as raw material. When the production is not carefully supervised, conditions may arise which are favourable for the formation of TCDD (Gribble, 1974), and then through effluents TCDD may enter the aquatic environment. Municipal incinerators can provide other sources of TCDD (Olie et al., 1977).

TCDD has a very low solubility in water (0.2 ppb) and is mainly adsorbed onto sediments where it is not readily available for microbial degradation; being very persistent it may accumulate in mud (Kearney et al., 1973; Ward and Matsumura, 1977).

In laboratory animals (Schwetz et al., 1973) TCDD is extremely toxic, having a $LD_{50}$ in the $\mu$g/kg range. It produces chloracne in man and rabbits (Kimmig and Schulz, 1957; Jones and Krizek, 1962) and causes several pathologic changes in the liver of different species (Gupta et al., 1973; Jones

and Butler, 1974; Vos et al., 1974). Edemas were reported in chickens (Schwetz et al., 1973), in mice (Vos et al., 1974) and in rat fetuses after maternal treatment (Khera and Ruddick, 1973).

Toxicity testing with salmonid fish and guppies has been carried out (Miller et al., 1973; Norris and Miller, 1974; Hawkes and Norris, 1977) but there are no reports concerning effects on early life stages. De Hullu and Poels (1976) observed a high toxicity for trout fry exposed to water of the river Rhine. The occurrence of severe edemas in exposed fry led to the speculation that TCDD, or similar contaminants, could have been the agent: although so far TCDD has not been detected in Dutch waters. This prompted us to study the effects of TCDD on early life stages of fish. The present study deals with the pike (*Esox lucius* L.)

## MATERIAL AND METHODS

A sample of TCDD (Lot number 851 : 144-II, 98.6% purity) was kindly supplied by Dow Chemical (Nederland) B.V. A stock solution of TCDD in dimethylsulfoxide (DMSO, BDH, AnalaR, purity 99%) at a concentration of 25 µg/ml was prepared, subsequent dilutions were made in acetone (BDH, Aristar, purity 99.8%).

Pike eggs and milt were obtained from clinically healthy pike caught by netting in the Pikmeer near Grouw, The Netherlands. The eggs were fertilized by the dry method (Huisman, 1975). After 5 min the eggs were divided into 5 groups of approximately 5000 eggs each and transferred to glass jars containing 1 l of tap water; 0.05 ml of TCDD in acetone was added to four jars to provide concentrations of 10.0, 1.0, 0.1 (ng/l) and 0.0 ppt TCDD; one group remained untreated. After transportation to the laboratory, which took 2 h, the eggs were transferred to glass tanks, containing 20 l of tap water (pH 6.9, hardness 94—102 ppm $CaCO_3$). TCDD—acetone solutions and acetone were again added, to provide the same concentrations as used in the glass jars. The medium was changed after 48 h, and after 96 h was replaced with tapwater which was then changed every 24 h. No control with DMSO was used. In other work (Helder, manuscript in preparation) it was demonstrated that concentrations of DMSO at the same level as used in this study (maximum 0.4 ppm) are not toxic.

The eggs were incubated on perforated aluminum trays suspended in tanks which were continuously aerated; water temperature was kept at 14 ± 0.5°C. After hatching, wooden boards were placed in the aquaria to which the fry became attached (Huet, 1975).

The fry were fed with live zooplankton and after development to the swimming stage all groups were reduced at random to 500 fish. Reduction of the 10-ppt group was not necessary because of the high mortality rate. For measuring length and light microscopy studies, 10 fishes were taken from each group, 2—3 times a week, anaesthetized in buffered tricaine methane sulphonate (MS 222®, Sandoz, Basel), fixed in Bouin Hollande for 24 h,

stored in 70% ethanol and after measurement of length they were embedded in Paraplast®. Transverse sections 7 μm thick were taken serially at distances of 100 μm and stained with Mayers haemalum and eosin. Clinical abnormalities and mortalities in all stages of development were recorded. For statistical analysis the *t*-test was used (Brown and Hollander, 1977).

## RESULTS

### *Clinical observations*

Embryonic development was considerably retarded in all TCDD-treated groups. Control groups hatched during the 8th and 9th day after fertilization, whereas the exposed groups hatched during the 10th and 11th day. Tail-hatching, i.e. hatching with the tail first, was observed for all TCDD-treated groups. In the 0.1 ppt group the incidence was less than 10 percent, in the 1 ppt group about 45 percent and in the 10 ppt group over 90 percent. Not a single case was observed in the controls. A number of tail-hatched fry were too weak to free themselves from the egg shells and died. Exposed fry were also less pigmented and smaller in size (Fig. 1). For a long period after hatching (Table 1) body lengths were significantly ($p < 0.05$) shorter than those of controls. In all groups, however, the transition to the stage of swimming fry occurred 15 days after fertilization.

Mortalities were recorded over three periods. Before hatching, the egg mortality was of the same order in all groups. In the yolk-sac fry stage, striking dose-related mortalities were observed which continued during the stage of swimming fry, although to a much smaller extent (Table 2).

Closely related to the mortality rates was the incidence of generalized edemas in the TCDD-treated fry. In the 0.1 ppt group it was less than 5

TABLE 1
BODY LENGTH (mm)[a] OF PIKE FRY AFTER 96-hours EXPOSURE TO TCDD

| Day after fertilization | Control | Acetone control | Dose TCDD | | |
|---|---|---|---|---|---|
| | | | 0.1 ppt | 1.0 ppt | 10.0 ppt |
| 11 | 9.0 ± 0.15 | 8.8 ± 0.13 | 8.5 ± 0.16[b] | 7.8 ± 0.20[f] | 7.3 ± 0.15[f] |
| 15 | 11.6 ± 0.16 | 11.4 ± 0.16 | 10.7 ± 0.21[e] | 10.1 ± 0.10[f] | 9.3 ± 0.15[f] |
| 18 | 13.3 ± 0.15 | 13.1 ± 0.10 | 12.8 ± 0.13[c] | 12.3 ± 0.15[f] | 11.3 ± 0.15[f] |
| 21 | 14.5 ± 0.17 | 14.4 ± 0.22 | 13.9 ± 0.10[d] | 13.6 ± 0.16[e] | 12.8 ± 0.20[f] |
| 23 | 14.6 ± 0.26 | 15.0 ± 0.15 | 14.1 ± 0.44 | 14.4 ± 0.29 | 13.6 ± 0.30[c] |

[a] Mean value ± S.D., 10 animals per sample.
[b] $p < 0.050$.
[c] $p < 0.025$.
[d] $p < 0.010$.
[e] $p < 0.005$.
[f] $p < 0.001$.

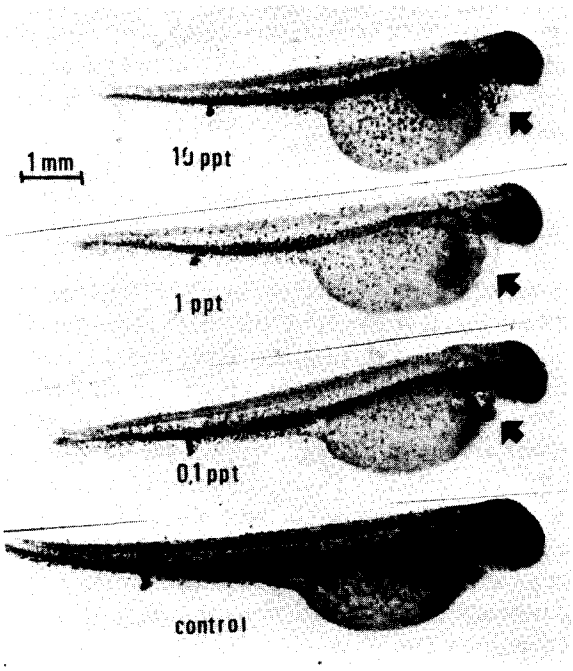


Fig. 1. Appearance of pike yolk sac fry, exposed to different concentrations of TCDD, 11 days after fertilization. Arrows indicate the onset of edema.

TABLE 2
MORTALITIES DURING EARLY LIFE STAGES OF PIKE AFTER 96 HOURS EXPOSURE TO TCDD

| | Control | | | Acetone-control | | |
|---|---|---|---|---|---|---|
| | a | b | c | a | b | c |
| Egg | 1671/5000 | 33.4 | 33.4 | 1713/5000 | 34.3 | 34.3 |
| Yolk sac fry | 303/3279 | 9.2 | 39.5 | 457/3237 | 14.0 | 43.5 |
| Swimming fry | 10/420 | 2.9 | 41.1 | 11/420 | 2.6 | 45.0 |

Dose TCDD

| 0.1 ppt | | | 1.0 ppt | | | 10.0 ppt | | |
|---|---|---|---|---|---|---|---|---|
| a | b | c | a | b | c | a | b | c |
| 1750/5000 | 35.0 | 35.0 | 1671/5000 | 33.4 | 33.4 | 1714/5000 | 34.3 | 34.3 |
| 662/3200 | 14.2 | 48.8 | 1609/3279 | 48.8 | 65.9 | 3061/3236 | 94.1 | 61.8 |
| 23/420 | 5.8 | 51.2 | 27/420 | 6.4 | 68.0 | 108/135 | 80.0 | 98.9 |

a = Number death/total. b = percentage death in given stage; e = cumulative mortality in percent.

percent, in the 1.0 ppt group 40 percent, and in the 10 ppt group nearly all fry developed severe edemas. No edematous fry were observed in the control groups.

*Histopathology*

In the clinically edamatous fry the histopathologic changes included edemas and haemorrhages, alterations of blood vessel walls and liver damage. These changes always occurred together.

Extensive accumulations of protein-containing fluid could be observed throughout the body, particularly in the soft connective tissue of the hypodermis, behind the eyes and between fibres of the hypaxial musculature. Consequently, the abdominal wall was completely disorganized and compared with the normal state its thickness was increased up to 3 times its normal thickness (Fig. 2). The pericardial and abdominal cavities were also extremely dilated due to the excess of fluid (Fig. 3) and in severe cases the fluid contained blood cells. Many capillaries and smaller bloodvessels in the edematous regions were disrupted and the nuclear size of endothelial cells was slightly increased. In the heart, the endothelial linings of both atrium and ventricle were discontinuous and occasionally red blood cells could be observed beneath the endothelium.

In pathological changes found in the liver, two stages could be distinguished. In the first, there was a severe dilation of the liver sinusoids and nuclear size at the hepatocytes was slightly increased (Fig. 4). In the

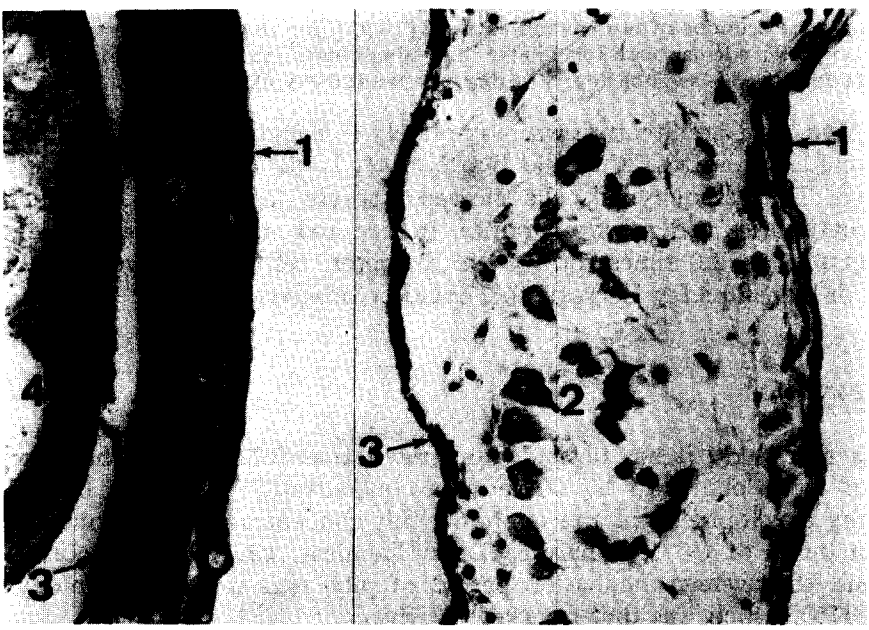


Fig. 2. Body walls of normal and of exposed (10ppt) pike fry. Note the extravasated bloodcells. × 360. 1 = cutis; 2 = musculature; 3 = peritoneum; 4 = yolk.

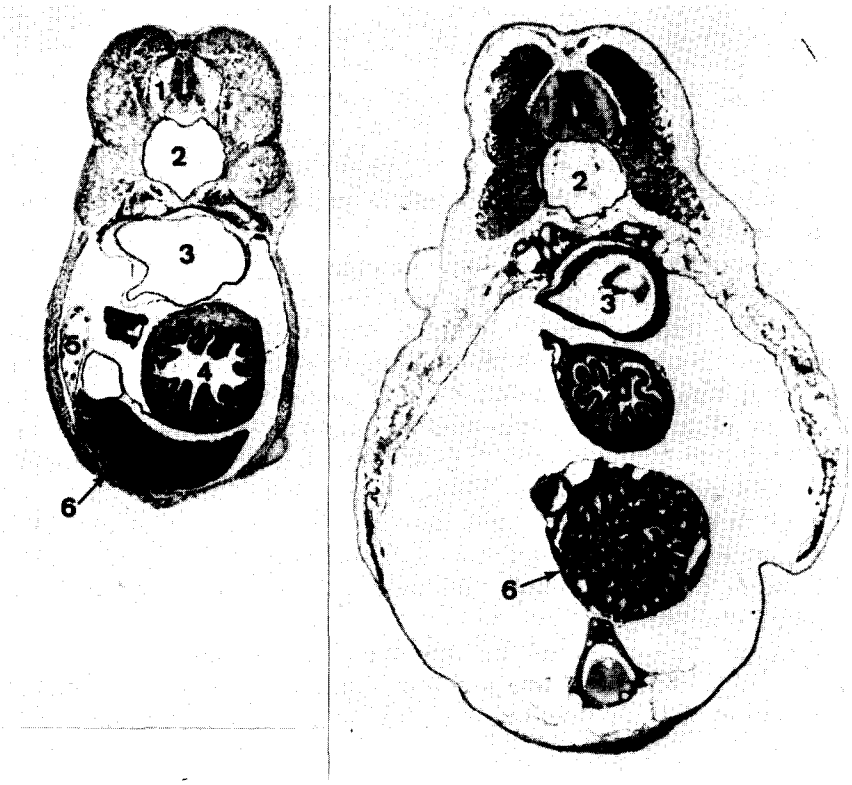


Fig. 3. Transverse sections of normal and exposed (10 ppt) fry 18 days after fertilization. Note the extreme edemas and the dilated liver sinusoids in the fry. 1 = spinal cord; 2 = chorda dorsalis; 3 = swimbladder; 4 = digestive tract; 5 = yolk; 6 = liver.

second stage, liver architecture was completely lost and the normal double-row lamina and rosettes (Elias and Bengelsdorf, 1952) (Fig. 5) were hardly recognised. The parenchymal cells showed a severe degeneration with pale staining cytoplasm and a wide variation in size and stage. Nuclei were enlarged up to 1.5—2.0 times the normal diameter, cell borders were badly outlined or lost and a few necrotic foci could be observed (Fig. 6).

## DISCUSSION

During this study it has been demonstrated that following 96 hour exposure of fresh pike eggs to TCDD at a concentration as low as 0.1 ppt retards the development of the embryos; this concentration is beyond the detection limits of GC/MS analysis (Dow Chemical, 1978). Under normal conditions, the average incubation period of pike eggs is 120 degree days (Huet, 1975), which was that required for the controls. The TCDD-exposed eggs had an incubation period averaging 147 degree days, that is a delay of about 23%. The appearance of tail-hatching is not directly associated with

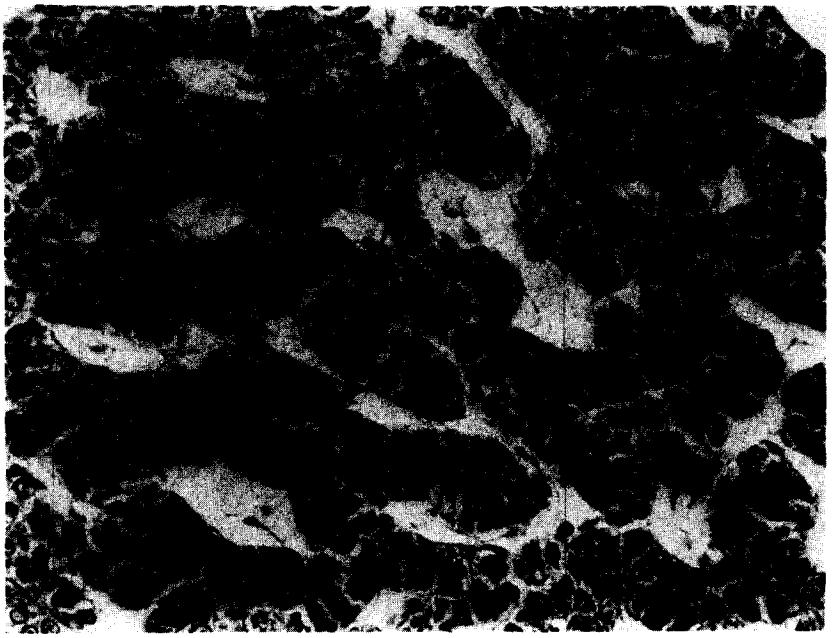

Fig. 4. Liver of pike fry exposed to 10 ppt TCDD, 16 days after fertilization. Parenchymal cells are slightly degenerated with minor nuclear enlargement, and dilated liver sinusoids. × 360.

Fig. 5. Normal liver of pike fry with rosettes and two cell layers thick lamina, 18 days after fertilization. × 480.

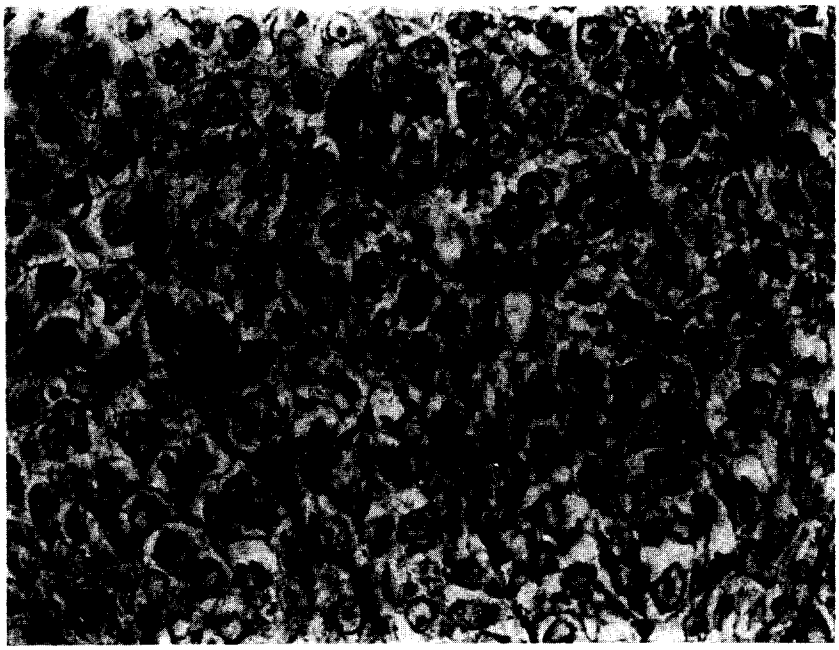

Fig. 6. Liver of pike fry exposed to 10 ppt TCDD, 18 days after fertilization. Severe degeneration of hepatocytes, with marked nuclear enlargement and complete loss of liver architecture. × 480.

the longer incubation periods, because this phenomenon was of the same order in all exposed groups. It is, however, related to the degree of underdevelopment of the pike fry. In fish culture, tail-hatching is generally considered to be a sign of weakness. Developmental retardation is also shown by the growth rates. These were significantly ($p < 0.05$) lower than the controls during the whole experiment, except for the 23rd day after incubation for the 0.1 and 1.0 ppt groups.

Mortality in the egg stage is not influenced by TCDD. A loss of 30—50 percent of pike eggs, because they are not fertilized, is considered normal under hatchery conditions (Huet, 1975). The high mortality among yolk sac fry is partly associated with the inability of tail-born fry to free themselves from the egg shells, but to a much higher degree with the occurrence of generalized edemas. Edema formation is a consistent feature of TCDD-poisoning (Khera and Ruddick, 1973; Schwetz et al., 1973; Vos et al., 1973). Also the presence of hydropericardium and degenerative changes of bloodvessels are in concordance with previous reports, concerning animals fed with fat-containing TCDD (Allen, 1964; Allen and Carstens, 1967).

Pathological changes that were found in the liver, i.e. focal necrosis and degeneration with pleiomorphism of parenchymal cells and nuclear enlargement, have also been observed in mammalian laboratory animals (Gupta et al., 1973; Jones and Butler, 1974). However, multinucleate hepatocytes, as observed in mice (Vos et al., 1973; Jones and Greig, 1975) were not

found during the course of this study. Disturbances of liver architecture have been described for monkeys (Allen and Carstens, 1967). The methods used in the present study were inadequate for detecting thymus atrophy, as is observed in mammalian species (Gupta et al., 1973; Vos et al., 1973) and in juvenile rainbow trout (Helder, in preparation). There was no sign of fin erosion (Norris and Miller, 1974). The extreme toxicity of TCDD to early life stages of pike may be due to the high lipophilia of the compound. The lipophilia of the TCDD, calculated according to the method of Rekker (1977) has a value of 7.1 expressed as log *P* octanol. This property could probably account for the high mortality in the yolk sac fry stage, during which the resorption of TCDD-containing yolk is increasing rapidly.

This study reported here may encompass the most vulnerable part of the life cycle of fishes and should have a wider application in the assessment of water quality.

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*Editor's comment*

The Dow Chemical Company has suggested that dioxins are ubiquitous in the environment, arise as a result of various combustion processes and are widespread. The processes invoked as providing sources of dioxins range from fossil-fuelled plants, cigarettes and charcoal-broiled steaks; in order to identify a common ground Dow put forward a theory of the trace chemistries of fire which requires the production of very small quantities of dioxins from a large number of processes at very low yields which is disputed by Professor Rappe, see Nature, London, Vol. 281, No. 5733, 25th October 1979, p. 619.

E. I. Hamilton

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