Temperature Selection in Brook Trout (*Salvelinus fontinalis*) Following Exposure to DDT, PCB or Phenol[1]

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Introduction

Synthetic chlorinated hydrocarbon compounds such as dichlorodiphenyltrichloroethane (DDT) and polychlorinatod biphenyls (PCB's) are now widely distributed throughout the biosphere. Because of similarities in their molecular structure, it is somewhat difficult to chemically distinguish DDT from a PCB and indeed, the presence of the latter may interfere with pesticide residue analysis (REYNOLDS 1969). The ubiquitous nature of these pollutants has raised many questions relating to their possible physiological and ecological effects (WURSTER 1969; GUSTAFSON 1970).

In the case of DDT, it has been shown that this chemical can interfere with behavioural thermoregulation (temperature selection) in fish (OGILVIE and ANDERSON 1965; GARDINER 1973), and the ecological implications of this finding have been considered by several authors (WURSTER 1969; WARREN 1971). An investigation of temperature selection in salmonids following exposure to a variety of chlorinated hydrocarbons revealed that although methoxychlor and several DDT analogs all shifted the selected temperature in a similar manner, the response was not altered by exposure to a PCB (PETERSON 1973).

In view of the chemical similarity between DDT and the PCB's, the apparent insensitivity of the temperature selection mechanism to PCB is of particular interest. Since Peterson reported on the effects of only one PCB dosage, however, the finding is not yet well documented. This paper therefore describes a more extensive comparison of the effects of DDT and a PCB on temperature selection in the brook trout. In addition, we also report on the effects of phenol. This chemical is of interest because as a pollutant it is dangerous to fish (JONES 1964), and in addition, its simple chemical structure is the basic component of both DDT and the PCB's.

1. Based on a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science at the University of Western Ontario.

Bulletin of Environmental Contamination & Toxicology, Vol. 11, No. 5 (c) 1974 by Springer-Verlag New York Inc.

MONS 084982

Methods

Underyearling brook trout (Salvelinus fontinalis, Mitchell) two to three months old were obtained from a commercial trout hatchery and held at 15°C for at least two weeks prior to experimental use. The fish were subjected to a 12L:12D photoperiod, and were fed Ralston Purina trout food once a day. Stock solutions of technical grade DDT (Nutritional Biochemical Corporation) were prepared in an acetone carrier, while the polychlorinated biphenyl (Monsanto Company, Arochlor 1254®) was dissolved in dimethylsulfoxide. Phenol (BDH Chemicals) was dissolved in water, and because of its volatile nature fresh solutions were prepared daily.

Prior to temperature gradient tests, the trout were fed for 10 minutes and then exposed for 24 hours to DDT (10 to 50 ppb), phenol (0.75 to 10 ppm), or PCB (25 to 100 ppm). For each exposure groups of five fish were placed in 2.5 litres of gently aerated water maintained at 15°C and containing 2.5 ml of the test chemical plus its carrier, or 2.5 ml of the carrier only. For each concentration tested two groups of experimental fish and two groups of control fish were so exposed and then tested simultaneously in a multichannel thermogradient device.

The temperature gradient apparatus has been described previously (OGILVIE and FRYER 1971). For these experiments four lanes were used, each 13 cm wide, 190 cm long, and 4 cm high. Each lane was divided into 20 positions 9.5 cm wide. Immediately after their exposure, each group of fish was placed in a separate lane and temperature selection in the control and experimental fish was determined simultaneously. Because some mortality occurred during exposure to the higher doses of the test chemicals, the number of experimental fish varied from 11 to 20.

Water depth in the apparatus was maintained at 1.8 cm and temperatures were recorded 5 mm above the floor of the control and experimental lanes with a YSI model 44TD telethermometer and a type 402 thermistor probe. Temperatures were found to be relatively linear along the length of the apparatus and ranged from about 4°C to 29°C. Gradient temperatures were recorded immediately before the introduction of the fish into the apparatus and at the completion of an observation period.

During a temperature selection experiment, the fish were viewed indirectly by means of a mirror

MONS 084983

suspended over the apparatus with the observer located behind a cardboard blind. After a one-hour equilibration period, the locations of the fish were recorded every two minutes for one hour.

The data was analyzed by arbitrarily dividing the thermogradient into 2°C intervals and determining the number of observations in each interval (see Fig. 4). Mean selected temperatures were then calculated and compared by Student's t-test. Differences were considered statistically significant when the P value was less than 0.05.

Results and Discussion

In the DDT experiments (Fig. 1) the mean temperatures selected by fish which had been exposed to the acetone vehicle only were relatively constant, ranging from 12.9 to 13.3°C. In the case of the DDT-exposed fish, however, the lowest dose used (10 ppb) significantly depressed the mean selected temperature, while the highest dose (50 ppb) significantly increased the selected temperature by more than four degrees. These results are clearly indicative of the biphasic dosage-response relationship reported previously for brook

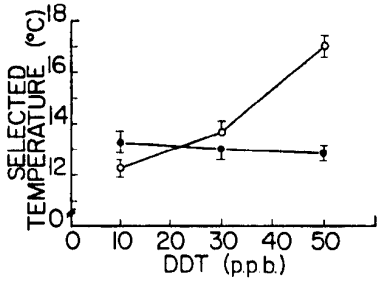

Fig. 1. Mean temperatures selected by brook trout following a 24-hour exposure to DDT in an acetone carrier (open circles) or acetone only (closed circles). Vertical bars denote ± 2 S.E.M.

MONS 084984

trout (GARDINER 1973) and also for Atlantic salmon (OGILVIE and ANDERSON 1965).

On the basis of the mortality which occurred during the DDT exposures, the 24-hour LD_{50} was estimated to be 54 ppb. No mortality occurred at 10 ppb, however, exposure to 30 ppb caused 20% mortality, and 50 ppb killed 45% of the fish in 24 hours. Thus, although the maximum shift in selected temperature was associated with relatively high mortality, the significant decrease in selected temperature produced by exposure to 10 ppb was not associated with mortality and therefore represents a truly sublethal effect.

It is evident from the phenol experiments (Fig. 2) that the control fish for the 10 ppm exposure selected a significantly higher temperature than the 0.75 and 5.0 ppm controls. The reason for this is unknown, however, we have observed similar day-to-day variation in control selected temperatures in other experiments (MILLER 1974), and the value of a multichannel thermogradient device in which control and experimental fish can be tested simultaneously is therefore apparent. Despite an unexplicable increase in the mean temperature selected by control fish, it is evident in Fig. 2 that exposure to phenol produced a consistant downward shift in selected temperature and further, this decrease was significant at doses of 7.5 and 10 ppm.

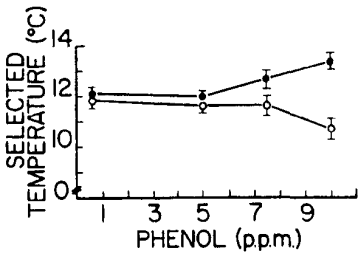

Fig. 2. Mean temperatures selected by brook trout following a 24-hour exposure to phenol (open circles). Control fish (closed circles) were held under comparable conditions for 24 hours prior to being simultaneously tested in the temperature gradient. Vertical bars denote ± 2 S.E.M.

MONS 084985

The 24-hour LD_{50} for phenol was estimated at 11.7 ppm and although the 10 ppm exposure produced 30% mortality, no deaths resulted from exposure to 7.5 ppm. As was the case with exposure to 10 ppb DDT, the temperature selection shift associated with this dose therefore clearly represents a sublethal effect.

As far as we are aware, there have been no previously published reports describing the effects of phenol on temperature selection in fish. It may be worthwhile to note, however, that we have obtained similar results with rainbow trout (MILLER 1974).

In contrast to the DDT and phenol results, 24-hour exposures to doses of PCB ranging from 25 to 100 ppm produced no mortality. The chemical similarity of DDT and PCB is well known (REYNOLDS 1969), and it is therefore somewhat surprising that no mortality was produced by PCB doses some thousandfold greater than the maximum DDT exposure used in these experiments. However, a separate mortality study using several species confirmed the fact that PCB is much less toxic to salmonids than DDT and in addition, the onset of mortality is somewhat delayed (MILLER 1974).

It is evident from Fig. 3 that all doses of PCB tested had no effect on the selected temperature of brook trout. Since these same exposures also produced no mortality, it may be argued that the fish were not subjected to a level of the chemical high enough to affect the mechanism of temperature selection. Since the maximum shifts in selected temperature produced by both DDT and phenol were associated with doses that also caused mortality, it seemed reasonable to increase the PCB exposure time until some mortality occurred, and then test the surviving fish in the temperature gradient.

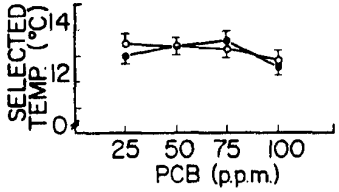

Fig. 3. Mean temperatures selected by brook trout following a 24-hour exposure to PCB in a dimethylsulfoxide carrier (open circles), or to dimethylsulfoxide only (closed circles). Vertical bars denote ± 2 S.E.M.

MONS 084986

Consequently, when 18 fish were exposed to 100 ppm. PCB for 48 hours, two fish died. The survivors were then tested in the gradient along with 19 controls. As can be seen in Fig. 4, a PCB exposure sufficient to cause mortality still had no effect on the selected temperature. In addition to the mortality noted above, a marked depression in locomotory behaviour was also observed during the 48-hour PCB exposure. Thus, while control fish swam actively about the exposure beaker, the PCB-exposed fish rested quietly on the bottom. Despite the chemical similarity between DDT and PCB, it therefore appears that PCB is much less toxic to fish than DDT and in addition, it does not appear to possess the ability to alter the thermoregulatory behaviour of fish as does DDT. This finding therefore confirms the preliminary observation by PETERSON (1973) who also failed to note any alteration in the temperature selected by PCB-exposed Atlantic salmon.

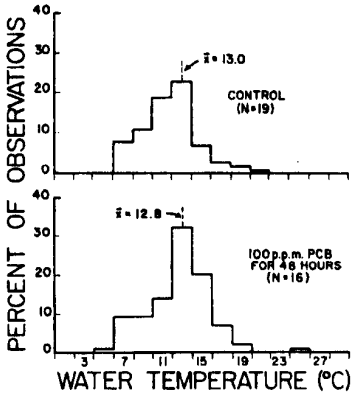

Fig. 4. Distribution of brook trout in a horizontal temperature gradient following a 48-hour exposure to 100 ppm PCB.

MONS 084987

References

GARDINER, D.R.: Pest. Biochem. Physiol. 2, 437 (1973).
GUSTAFSON, C.G.: Environ. Sci. Technol. 4, 814 (1970).
JONES, J.R.E.: Fish and river pollution. London: Butterworth 1964.
MILLER, D.L.: M.Sc. Thesis, University of Western Ontario, London, Canada (1974).
OGILVIE, D.M. and J.M. ANDERSON: J. Fish. Res. Bd. Can. 22, 503 (1965).
OGILVIE, D.M. and J.N. FRYER: Can. J. Zool. 49, 949 (1971).
PETERSON, R.H.: J. Fish. Res. Bd. Can. 30, 1091 (1973).
REYNOLDS, L.M.: Bull. Environ. Contam. Toxicol. 4, 128 (1969).
WARREN, C.E.: Biology and water pollution control. Philadelphia: W.B. Saunders 1971.
WURSTER, C.F. Jr.: Biol. Conservation 1, 123 (1969).

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Polychlorinated Biphenyl Residues in Maternal and Cord Blood in Tokyo Metropolitan Area

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INTRODUCTION

A number of reports indicate that PCBs are ubiquitously present in the biota of Japan (Tatsukawa and Isono, 1971, Mizutani et al, 1972, Hidaka et al, 1972, Kobayashi et al, 1972), although physiological significance is not known at this present level found in the fauna. An exposure to this substance for the human neonate starts at the commencement of breast feeding, however, the first exposure takes place as early as sometime during fetal stage, as indicated by the few incidences of "black babies" delivered by mothers with or without overt symptoms during the outbreak of PCB poisoning in Japan (Funatsu et al, 1971, Yamaguchi et al, 1971). Lipophilic chlorinated hydrocarbons such as DDT and PCB tend to remain in biota for years, once they are emitted to nature in substantial amount, even after termination of actual application. The production of PCBs was banned in 1972 in Japan and its use in the open system has also been forbidden. This report is a part of our project to monitor certain environmental chemicals found in the tissues of residents in Tokyo metropolitan area.

MATERIALS AND METHODS

Both maternal and cord blood was sampled at Tokyo University Hospital at the time of delivery during the period December, 1973 through February, 1974. Blood samples were taken in the blood container, pretreated with hexane and stored at 5°C in refrigerator until the time of analysis. For analysis of PCBs, we essentially followed the standard method recommended by the advisory committee for the Ministry of Health and Welfare (1972). Twenty g of whole blood in 50 ml centrifuge tube was shaken vigorously with 20 ml of ethanol. Five g of potassium hydroxide was added to this and heated at 90°C for one hour. After cooling, the content was extracted with three additional 20 ml portions of n-hexane. The hexane was washed three times with water, dried through the column of anhydrous sodium sulfate, and condensed

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Bulletin of Environmental Contamination & Toxicology, Vol. 13, No. 5 © 1975 by Springer-Verlag New York Inc.

in K-D condenser to the volume of 1 to 2 ml. The condensed sample was purified by letting it pass through the column consisting of 2 g of Florisin, 3 g of silica gel and anhydrous sodium sulfate. PCBs were eluated from the column with hexane and initial 180 ml of fraction was collected. The eluate was concentrated to 1 ml, shaken vigorously with 1 ml concentrated sulfuric acid and then hexane layer was buffered with potassium hydroxide pellets prior to gas chromatography.

The gaschromatograph used was Shimazu GC 5 AP equipped with ^{63}Ni Electron Capture Detector. The operative conditions for PCB analysis were as follows.

column dimensions	: 3 mm x 2m
column packing	: 2% OV - 1 on Gas Chrom. Q 80/100
column temperature	: 200°C
detector temperature	: 250°C
Nitrogen flow rate	: 60 ml/min.

The quantification of PCB residues followed the method provided by Ugawa (1973). Total blood lipids were determined by a modified method of Bragdon, i. e. 3 g of samples were treated twice in Folch's solvent with a dispersing mixer; the filtered solution was shaken with 0.05% sulfuric acid; the lower layer was evaporated to dryness and the residue was weighed.

RESULTS AND DISCUSSIONS

Our data is summarized in Table 1. The mean PCB level in adult blood (mother) obtained in our study (2.8 ppb) corresponds well to those reported by other workers in Japan (Inoue et al, 1974, Abe et al, 1974, Doguchi et al, 1975). On a whole basis, PCB levels in maternal blood are roughly threefold higher than those in cord blood. Because of its strong lipophilic tendency it was expected that PCBs were conveyed by the lipid fraction in the blood. Though the lipid levels in maternal blood were roughly twice as high as those in cord blood, the correction of the values to fat basis gave more or less similar results in terms of the concentration ratio between maternal and cord blood.

Gaschromatographic pattern of PCBs found in blood is fairly similar to the patterns noted in adipose tissues, namely closely resembling that of Kanechlor 500 and Kanechlor 600. The chlorine content of PCB residues in the present investigation is calculated roughly 53% which is very close to that of Kanechlor

MONS 084990

500 (54.6%).

It is noteworthy that gaschromatographic patterns of PCB residues found in the samples of mother-baby pair are almost identical (Fig. 1), and this suggests that the placenta permits rather indiscriminate transmission of the organochlorine compounds of closely related chemical structure.

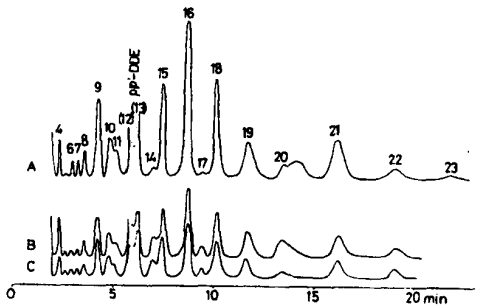

Figure 1: Gaschromatographic Patterns of PCB Residues.
A : Maternal blood
B,C : Cord blood of twins

Narafu (1973) noted an excellent correlation (0.972) as regards the PCB levels between maternal and cord blood, however we failed to see such relationship (-0.046 on a fat basis ; -0.018 on a whole basis). It appears that this issue remains unsettled in the cases of other organochlorine compounds such as BHC and DDT. (O'Leary et al, 1970, Yamagishi et al, 1972).

SUMMARY

PCB levels were determined with maternal and cord blood sampled at the time of delivery. All of the twentyfour mothers selected for this experiment are residents of Tokyo Metropolitan area. Gaschromatographic

MONS 084991

TABLE 1

PCB Levels of Maternal and Cord Blood

Case	Wet Basis (ppb)		Fat Basis (ppm)	
	M.	C.	M.	C.
1	3.5	---	0.42	---
2	2.6	1.5	0.18	0.31
3	2.0	0.9	---	0.23
4	3.3	---	0.35	---
5	3.9	0.3	0.41	0.06
6	4.5	1.6	0.53	0.33
7	1.6	0.7	0.25	0.13
8	2.4	0.7	0.30	0.14
9	2.1	0.9#	0.23	0.22#
		0.6#		0.16#
10	4.2	0.6	0.57	0.17
11	0.3	1.4	0.05	---
12	3.0	0.9	---	0.22
13	1.8	1.2	0.29	0.24
14	1.4	0.8	0.19	0.13
15	1.0	0.4	0.14	0.06
16	1.9	0.8	0.28	0.25
17	2.9	1.3	0.39	0.24
18	---	1.2	---	0.36
19	3.6	1.2	0.43	0.17
20	---	0.8	---	0.22
21	2.6	3.3	0.65	0.35
22	7.6	0.3	0.80	0.08
23	1.8	1.6	0.23	0.49
24	3.5	0.8	0.43	0.21
Av.	2.8	1.1	0.36	0.22
S. D.	1.5	0.6	0.18	0.10

#Twins

MONS 084992

patterns of PCBs in cord blood closely resembled those of mothers. The cord blood in general showed lower PCB levels on both whole and fat basis than those in the maternal blood. No correlation was noticeable on statistical basis as regards the concentrations of PCBs between the cord blood and maternal blood. Our data suggests that the passage of PCBs through the placenta follows general patterns observed in other organochlorine insecticides.

ACKNOWLEDGEMENT

We are indebted to Mr. K. Ohkawa for his technical assistance and to Mrs. S. T. Ohi for her useful critical discussions and typing of this paper.

REFERENCES

Abe, J. et al.: Jap. J. Hyg. 29, 93 (1974)

Doguchi, M. et al.: Bull. Environ. Contam. Toxicol. 13, 57 (1975)

Funatsu, I. et al.: Fukuoka Igaku Zasshi, 62, 139 (1971)

Hidaka, K. et al.: J. Food Hyg. Soc. Japan, 13, 227 (1972)

Kobayashi, H. et al.: ibid. 13, 523 (1972)

Inoue, Y. et al.: Jap. J. Hyg. 29, 92 (1974)

Mizutani, T. et al.: Igaku no Ayumi, 81, 666 (1972)

Narafu, C.: Rep. Aichi. Prefect. Inst. Pub. Health, 23, 1 (1973)

O'Leary, J. A. et al.: Amer. J. Obster. Gynec. 107, 65 (1970)

Tatsukawa, R. and Isono, N.: Shizen, 26, 30 (1971)

Ugawa, M. et al.: J. Food Hyg. Soc. Japan, 14, 415 (1973)

Yamagishi, T. et al.: Rinsho Eiyo, 41, 209 (1972)

Yamaguchi, A. et al.: Fukuoka Igaku Zasshi, 62, 117 (1971)

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