Polychlorinated Biphenyls (PCB) Solubilized in Water by Nonionic Surfactants for Studies of Toxity to Aquatic Animals

by V. ZITKO
Fisheries Research Board of Canada, Biological Station
St. Andrews, N. B.

PCB have been detected in marine animals in Sweden, England, USA, and The Netherlands (1,2,3). Knowledge about the toxicity of PCB to wildlife is limited. PCB are inducers of hepatic enzymes that hydroxylate steroids in birds (1) and a latent toxicity of PCB to grasshoppers (*Chorthippus brunneus*) at ecdysis has been suggested (4). No data on the toxicity of PCB to aquatic animals have been published.

The solubility of PCB in water is very low and difficulties are encountered in dosing PCB in experiments with aquatic animals. PCB-in-water emulsions, prepared by dilution of PCB, dissolved in an organic solvent, with water or by mechanical dispersing of PCB in water, coagulate on standing and contain PCB particles varying greatly in size. The results of toxicity tests using such PCB-in-water emulsions are difficult to reproduce and may not indicate the true toxicity of PCB. A PCB-in-water emulsion may display different degrees of toxicity or appear nontoxic depending on the size of PCB particles in the emulsion. A more stable and homogeneous PCB-in-water system was therefore sought. This paper describes the solubilization of PCB in water by nonionic surfactants, the determination of concentration of the solubilized PCB by UV spectrophotometry and fluorescence, some properties of aqueous PCB solutions, and preliminary results on the toxicity of PCB to Atlantic salmon (*Salmo salar*) parr*.

EXPERIMENTAL

Commercial PCB preparations containing 21 and 54% chlorine (Aroclor 1221 and Aroclor 1254) were used. Ethylene oxide adduct (9 ethylene oxide units) of lauric acid, ethylene oxide adduct (8 ethylene oxide units) of 2,6,8-trimethyl-4-nonanol (both preparations from the Surfactant Kit, Chem Service, Media, Pa.), and a commercial emulsifier preparation Corexit 7664 (Enjay Chemical Co.) were used to solubilize PCB in water. Typically, Aroclor (500 mg) was dissolved in Corexit 7664 (9500 mg) and the volume was adjusted to 50 ml with

---

*A more detailed presentation of all results is given in (5). Toxicity of PCB, solubilized by Corexit 7664, to *Gammarus oceanicus* has been recently described (6).

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 3, 1970, published by Springer-Verlag New York Inc.

distilled water. St. Andrews tap or sea water was used for further dilutions.

UV and fluorescence spectra were recorded on a Beckman DK-2A and on a Perkin-Elmer MPF-2A instrument respectively. Gas chromatography was carried out on a Varian 600D instrument equipped with a 250 mC tritium electron capture detector, using a 5 ft, 1/8 in. glass column, operated at 180°C and containing 4% SE-30 on Chromosorb W, 100-120 mesh.

To determine the solubility of PCB in water, Aroclor (1.5 ml) was added to fresh or sea water (50 ml) in a Waring blendor and the mixture was homogenized for 10 min at room temperature. The resulting emulsion was centrifuged for 30 min at 30,000 g (5°C) and the concentration of Aroclor in the clear supernatant was determined by spectrophotometry. A part of the supernatant was extracted with hexane and the extract was analyzed by gas chromatography.

Toxicity tests with Atlantic salmon parr (average weight 4.3 g, average length 7.2 cm) were carried out at 15°C in 3 liter Erlenmeyer flasks containing 2 liters of Aroclor-Corexit solutions. Each flask was aerated by bubbling air through a glass tube immersed in the solution. Two flasks were used at each Aroclor concentration: one containing 2 fish, the other without fish, serving as a blank. Tests were terminated after 192 hours. At this time fish were still alive in solutions of Aroclor 1254 and Aroclor 1221, initial concentration 0.9 and 2.5 and 0.9 mg/l, respectively (Fig. 1,2). Samples (10 ml) were withdrawn from the flasks at different times and the concentration of Aroclor was determined by spectrophotometry or by fluorescence.

RESULTS

Solubility of PCB in water. A fractionation according to the degree of chlorination takes place on breaking Aroclor-in-water emulsions (no surfactant added) by centrifugation. The dissolved fraction is richer in lower chlorinated biphenyls than the original preparation. The quantitative composition of Aroclor 1254 and of the mixture of biphenyls recovered from the supernatant are given in Table 1. The difference between the original Aroclor 1254 and the mixture of biphenyls in the soluble fraction causes some uncertainty in the determination of the solubility of Aroclors in water. The solubility of Aroclor 1254 is 2–3 mg/l in fresh and 1–1.5 mg/l in sea water. This batch of Aroclor 1254 contains a relatively high amount of lower chlorinated biphenyls. The solubility of another batch of Aroclor 1254, containing only traces of lower chlorinated biphenyls, is 0.3–0.5 mg/l in both fresh and sea water. The solubility of Aroclor 1221 is 5.0 and 3.8 mg/l in fresh and sea water, respectively.

TABLE 1

Quantitative composition of a batch of Aroclor 1254 and of its water-soluble fraction.

	Relative peak area in order of increasing retention time							
Aroclor 1254	0	0.2	0.1	0.1	3.8	1.2	1.3	10.3
Sol.fraction	0.2	0.4	0.2	0.2	1.3	0.6	0.7	8.6
Aroclor 1254	16.0	22.0	15.7	13.6	11.1	2.7	1.9	
Sol.fraction	14.4	17.5	18.7	16.5	15.7	2.7	2.3	

Solubilization of PCB. A solution of PCB in ethylene oxide adduct of lauric acid (weight ratio 1:20), containing isopropanol (0.6 g/gPCB), yields on dilution with water a clear solution. Acetone (1.5 g/gPCB) may be used instead of isopropanol but the same amount of dimethyl sulfoxide is not effective. A solution of PCB in ethylene oxide adduct of 2,6,8-trimethyl-4-nonanol (weight ratio 1:10) similarly requires isopropanol (0.6 g/gPCB) to yield a clear aqueous solution. A solution of PCB in the same surfactant (weight ratio 1:20) yields, even without isopropanol, a clear solution when diluted with water. Corexit 7664 contains isopropanol and a solution of PCB in Corexit 7664 (weight ratio 1:19) gives a clear solution on dilution with water.

Determination of PCB solubilized in water. UV spectrum of Aroclor 1221, solubilized by Corexit 7664, has a maximum at 247 nm, log Σ 4.18 (Aroclor 1221 in ethanol: 247 nm, log $\Sigma = 4.23$). Absorbance increases steadily towards shorter wavelengths in solutions of Aroclor 1254 containing Corexit 7664; at 250 nm log $\Sigma = 3.97$ (in ethanol log $\Sigma = 4.00$ at 250 nm). Lambert-Beer's law is obeyed by both Aroclors in the concentration range examined (0.5 – 10 mg/l); concentration of Aroclor 1221, c (mg/l) = 12.6(A247); of Aroclor 1254, c = 35.1(A250); (A247), (A250) = absorbance in 1 cm cell at 247 and 250 nm, respectively. Fluorescence excitation maximum of both Aroclors is at 270 nm, fluorescence emission maximum is at 316 and 320 nm for Aroclor 1221 and 1254, respectively. Relative fluorescence at 320 nm is a linear function of concentration up to 0.5 and 4.0 mg/l and the detection limits are approximately 0.025 and 0.25 mg/l for Aroclor 1221 and 1254, respectively.

Variations occur between different batches of Aroclors with the same declared chlorine content; both spectrophotometry and fluorescence require calibration using the particular batch of Aroclor which is to be determined.

Adsorption of PCB. In water solubilized PCB are strongly adsorbed by plastic surfaces such as polyethylene sheets and tubes.

Toxicity of PCB to Atlantic salmon parr. Aroclors solubilized by Corexit 7664 were used because of the known low toxicity of the latter*. The experiments were carried out primarily to determine the behaviour of solubilized PCB in toxicity tests with aquatic animals. Two fish were tested at each concentration, the toxicity data are therefore only preliminary.

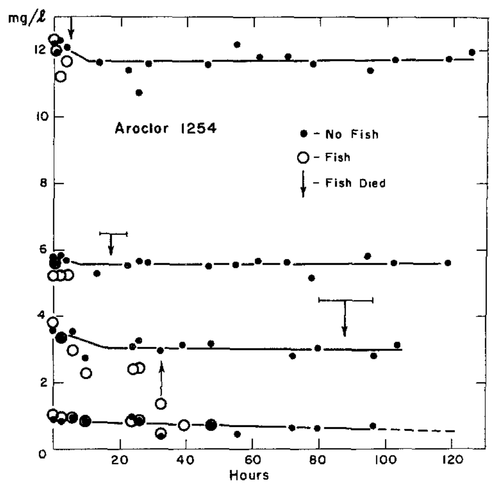

Figure 1. Concentration of Aroclor 1254 in the bioassay.

---

*No mortality of Atlantic salmon parr exposed to 500 mg/l of Corexit 7664 was observed in 96 hours (7).

Concentration of Aroclor 1254 in the experiments is presented in Figure 1. The concentration in flasks without fish remained practically constant. Decrease in concentration caused by uptake of Aroclor by fish is noticeable with the exception of the lowest concentration. Aroclor 1254 is lethal to Atlantic salmon parr at concentrations higher than 2 mg/l. The concentration of Aroclor 1221 decreased steadily during the experiment (Fig. 2). The uptake of Aroclor 1221 by fish is pronounced at the two intermediate concentrations tested. Aroclor 1221 is, under the described conditions, lethal to Atlantic salmon parr at initial concentrations higher than 2 mg/l. It has been demonstrated in separate experiments that the losses of Aroclor 1221 are caused by its volatility on aeration. According to Figure 1 Aroclor 1254 appears to be non-volatile. However, PCB were detected in the air of a cold room (5°C) used for tests with Aroclor 1254.

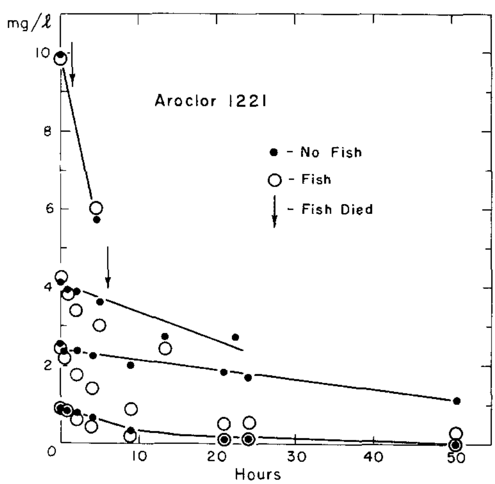

Figure 2. Concentration of Aroclor 1221 in the bioassay.

DISCUSSION

The determined levels of solubility of PCB in water were reached by very vigorous dispersing of PCB in water and it would be difficult to scale up this procedure to prepare a large volume of the PCB solution.

As indicated by the toxicity data, lethal levels would be reached only in the case of Aroclor 1221. Because of the solubility differences between higher and lower chlorinated biphenyls, mainly the toxicity of the latter would be tested even in solutions prepared by dispersing Aroclors with higher chlorine content, in water. The solubilization of PCB in water by relatively nontoxic nonionic surfactants is a reproducible and convenient method for dosing PCB in experiments with aquatic animals. The whole PCB preparations are solubilized and the stock solution of the solubilized PCB may be used in running-water toxicity tests. Concentration of the solubilized PCB can be readily monitored by spectrophotometry or by fluorescence at a rate of 20–30 analyses per hour; a continuous monitoring is also possible.

From the point of view of optical properties there is no difference between PCB solubilized in water by nonionic surfactants and true solutions of PCB in ethanol. The mechanism of the solubilization is as yet unknown.

The presence of Corexit 7664 may enhance the toxicity of PCB by providing better contact between fish and PCB than a mechanical dispersion of PCB in water or a dilution of PCB dissolved in an organic solvent. It is not known whether Corexit 7664, at the concentrations used, affects the permeability of fish body surface to PCB.

The preliminary tests indicate that PCB may be less toxic to Atlantic salmon parr than chlorinated hydrocarbon pesticides.

Inhalation of PCB represents a health hazard (8). Because of the volatility of PCB, experiments must be carried out in well ventilated facilities.

REFERENCES

1. R. W. RISEBROUGH, P. RIECHE, D. B. PEAKALL, S. G. HERMAN and M. N. KIRVEN, Nature 220, 1098 (1968).

2. J. H. KOLMAN, M. C. TEN NOEVER DE BRAUW and R. H. DE VOS, Nature 221, 1126 (1969).

3. S. JENSEN, A. G. JOHNELS, M. OLSSON and G. OTTERLIND, Nature 224, 247 (1969).

4. F. MORIARTY, Entomol. Exp. Appl. 12, 206 (1969); CA 71, 89051 (1969).

5. V. ZITKO, Fisheries Research Board of Canada MS Report No. 1083, Polychlorinated biphenyls: determination by optical methods, solubility and solubilization in water, preliminary results on toxicity to salmon (April 1970).

6. D. J. WILDISH, Fisheries Research Board of Canada MS Report No. 1084, Polychlorinated biphenyls in sea water: Bioassay of *Gammarus oceanicus* (May 1970).

7. V. ZITKO and W. G. CARSON, Fisheries Research Board of Canada MS Report No. 1043, Bunker C oil. Dispersibility in water by Corexit and Xzit at different temperatures (October 1969).

8. HANDBOOK OF ANALYTICAL TOXICOLOGY, I. Sunshine, Editor, p 614 (1969), The Chemical Rubber Co., Cleveland.