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Mutagenicity, Acute Toxicity, and Bioaccumulation Potential of Six Chlorinated Styrenes

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Environmental octachlorostyrene (OCS) was first found in birds from the Rhine River and the Netherlands coastal area (Ten Noever de Braw and Koeman 1972/73). The compound was later identified in fish and heron from the Great Lakes (Kuehl et al. 1976; Reichel et al. 1977). In the marine environment OCS has been identified together with hexa- and heptachlorostyrene as a local pollutant in the Norwegian fjord Frierfjorden (Lunde and Baumann Ofstad 1976; Baumann Ofstad et al. 1978) and recently OCS was detected in fish, water and sediments of the North Sea and adjacent estuaries (Ernst et al. 1984).

The local source of the Norwegian chlorinated styrene pollution is magnesium production and chlorinated styrenes have been identified in samples of water, sediment, fish, birds' eggs and in blood samples from factory workers and humans with a high consumption of fish (Baumann Ofstad 1978; Lunde and Bjørseth 1977).

So far little is known about the potential biological effects of chlorinated styrenes. The aim of the present study was to investigate the acute toxicity towards aquatic organisms, the mutagenicity and the bioaccumulation potential of some penta-, hexa-, hepta- and octachlorostyrenes.

MATERIALS AND METHODS

The six chlorostyrenes studied are presented in Table 1. The octachlorostyrene sample was a gift from O. Bøckman, Norsk Hydro a.s while the other isomers were synthesized by Kolsaker et al. (1983).

Nitocra spinipes, Boeck (Crustacea) used for the acute toxicity testing is a typical brackish water organism, playing an important role in the food chains of the Baltic Sea. It is found in coastal regions in Europe, Asia, Africa and North America and often occurs in substantial amounts, especially on sand bottoms, interstitially, or free in the phytal. Nitocra tolerates salinities from marine to almost limnic conditions. The length of the adult animal is about 0.6-0.8 mm. It is simple to rear in the laboratory (Bengtsson 1978; Gopalan 1977) and the development from egg to the adult stage takes about two weeks.

The acute 96-h toxicity test with Nitocra (Bengtsson 1981) was performed at room temperature (20-22° C) under static conditions in 15 ml test tubes containing 10 ml of test water. The physico-chemical characteristics of the natural brackish water used were close to constant in the experiments: salinity 7 o/oo, alkalinity 1.5 megv l^{-1}, pH 7.8. The water had been previously heated to 80° C and filtered through a paper filter. Acetone was used as solvent for the test compounds and added to a concentration of 500 mg/L in the test tubes. Each concentration of the test compounds was tested in duplicate. The test solutions were not aerated.

The calculation of the 96-h LC_{50} values and the corresponding 95 % confidence limits were made with a data program for probit analysis according to a Statistical Analysis System Program (SAS) at the Stockholm University Computer Center.

The potential mutagenic activity of the compounds was examined in the Salmonella/ microsome assay as described by Ames et al. (1975). The strains S. typhimurium TA98 and TA100 were used with and without metabolic activation with a liver homogenate (S9) prepared from Aroclor-induced rats. The compounds were solubilized in dimethylsulfoxide to a final concentration of 10 or 20 mg/mL, and tested in the dose range from 2 µg to 1, 2 or 4 mg per plate, depending on the amount of compound available.

Reversed phase thin layer chromatography (HPTLC) was used for the estimation of the partition coefficients P_{ow} of the chlorostyrenes in an octanol - water system according to the method described by Renberg and Sundström (1979). The stationary nonpolar phase was chemically bounded C_8 carbon chains on silica gel (RP-8, Merck 13725). As eluting solvent, distilled water:acetone (pa) (30:70 v/v), was used. Four substances, with known partition coefficients were used as internal standards. The spots were visualized in UV light. Log $(1/R_F-1)$ -values were calculated and plotted in a diagram against log P_{ow} of the known internal standards. From the equation of the linear standard curve the log $P_{ow:i}$ values for the chlorostyrenes were calculated.

RESULTS AND DISCUSSION

The Nitocra test, regularly used at the Brackish water toxicology laboratory, is regarded convenient for studying the acute toxicity of synthesized compounds only available in small quantities. Less than 1 mg of the substance was needed in the test with transhexachlorostyrene (96-h LC_{50} value >10 mg/L).

The results from the Nitocra tests are shown in Table 1. Five of the chlorostyrenes are highly toxic with LC_{50} values between 34 - 150 µg/L. Transhexachlorostyrene was considerable less toxic than the others with a LC_{50} value >10 mg/L. Due to the low solubility of the compounds the highest possible test con-

centration was 15 mg/L. In comparison it should be mentioned that the acute toxicity to Nitocra spinipes for p,p'-DDT and pentachlorophenol have been shown to be 30 and 270 µg/L respectively (Lindén et al. 1979).

Table 1. Acute toxicity of six chlorostyrenes, to the harpacticoid Nitocra spinipes.

Chlorostyrenes	Purity (%)	Nitocra spinipes 96-h LC_{50} (mg/L) * (21 ±1°C)
Pentachlorostyrene	99.5	0.056 (0.049-0.068)
Transhexachlorostyrene	98.8 + 99.7	> 10
Transheptachlorostyrene	96.0	0.15 (0.12-0.19)
Cisheptachlorostyrene	97.6 + 95.8	0.034 (0.030-0.039)
ββ-heptachlorostyrene	90	0.038 (0.032-0.045)
Octachlorostyrene		0.068 (0.032-0.121)

*LC_{50} values with 95 % confidence limits are given.

No data are found in the literature concerning the toxicity of chlorostyrenes to aquatic organisms.

Studies of the acute toxicity of octachlorostyrene against fish are currently carried out at the Brackish water toxicology laboratory.

Chu et al. (1982a) have recently performed an investigation on the acute and subacute toxicity of OCS in the rat. The results suggest that OCS can produce biochemical and histological changes in rats after administration of a single oral dose and/or when fed in the diet. OCS was found to possess some toxic properties similar to those reported for hexachlorobenzene and the study indicated a maximum no-effect level of 0.5 ppm for histological changes in the liver and thyroid of the rats.

None of the chlorostyrenes were mutagenic towards the strains TA98 or TA100 in the dose range tested as neither of the compounds showed a dose-related increase in the number of revertants or a doubling of revertant colonies compared to the control.

Other chlorinated compounds such as several PCB's, hexachlorocyclohexanes and hexachlorobenzene also lack mutagenic activity in bacterial short-term tests. It has been suggested that this partially may be due to a low rate of metabolism by S9 (Rinkus and Legator 1979).

To study the bioaccumulation and elimination of chemicals in biota biological experiments are usually performed. One of the fac-

tors which determine the degree of bioconcentration of organic compounds in biota is the lipophilicity, usually estimated from the partition between 1-octanol and water P_{ow}. The partition coefficient is therefore used to predict the bioconcentration potential of substances of which the uptake mainly depends on the lipophilicity.

The use of reversed phase thin layer chromatography for the estimation of P_{ow} has been suggested to be a convenient screening parameter for the prediction of the behaviour of organic chemicals in the environment (Renberg and Sundström 1979; Renberg et al. 1980). Good correlation has been shown to exist between the bioconcentration factor and log $(1/R_F-1)$ for some chemicals.

Veith et al. (1979) calculated a correlation coefficient of 0.897 for the bioconcentration factors obtained from fish tests and the P_{ow}'s for the 55 chemicals used in the bioassays.

The calculated log $P_{ow:i}$ determined on the reversed phase HPTLC C_8-plates for the six chlorostyrenes are listed in Table 2. The results indicate a very high bioaccumulation potential for the acutal chlorostyrenes ranging between 6.86 - 7.68. Octachloro- and transheptachlorostyrene are the most lipophilic and transhexa- and cisheptachlorostyrenes are less lipophilic compounds. As a comparison Renberg and Sundström (1980) have reported values of 6.19 for p.p'-DDT and 2,4',5-trichlorobiphenyl.

Table 2. The logaritmic values of the partition coefficients calculated from chromatographic data from reversed phase HPTLC-C_8 plates

Chlorostyrenes	Calculated log $P_{ow:i}$
Pentachlorostyrene	6.93
Transhexachlorostyrene	6.86
Transheptachlorostyrene	7.14
Cisheptachlorostyrene	6.89
ββ-heptachlorostyrene	7.00
Octachlorostyrene	7.68

Ernst et al. (1984) have found high concentrations of octachlorostyrene in fish and sediment compared to water in the German Bight, and a possible high degree of bioconcentration and adsorption potential of this compound is discussed. Our results from the estimation of the partition coefficients verify a considerable accumulation potential in lipophilic tissues of OCS.

In a recent study Norheim (personal communication) investigated the depuration of OCS and hexachlorobenzene (HCB) in fish. A mix-

ture of the two compounds were intraperitoneally injected into rainbow trout and the concentration of the two compounds was monitored during a 6 months elimination phase. The apparent half-life of octachlorostyrene in the fish liver was nearly twice as long as for hexachlorobenzene, 143 and 81 days respectively.

The investigations performed so far in the Frierfjord indicate a slow metabolization of chlorostyrenes in the marine environment (Lunde and Baumann Ofstad 1976; Baumann Ofstad et al. 1978).

Chu et al. (1982b) have studied the tissue distribution, metabolism and excretion of octachlorostyrene in the rat. They found that OCS was very stabel with a slow elimination and/or metabolism in rats. Low concentrations of the metabolites pentachlorodichlorophenylacetic acid and a heptachlorostyrene isomer were observed.

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