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Porphyrinogenic Action of Tetrachloroazobenzene

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Some environmental identified halogenated aromatics possess porphyrinogenic properties. Among them are halogenated biphenyls, terphenyls, naphtalenes, dibenzodioxins, benzenes and some other related products (STRIK et al. 1980).

The purpose of this study was to determine the porphyrinogenic potential of 3,3',4,4'-tetrachloroazobenzene (TCAB). Chlorinated azobenzenes are structurally similar (isosteric) to the chlorinated dibenzodioxins. 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) is a very potent inducer of hepatic porphyria (GOLDSTEIN et al. 1973) and produces chloracne (POLAND et al. 1971). Recently, HILL et al. (1981) demonstrated in the rabbit ear test that the chloracnegenic potential of TCAB was in the same range of TCDD.

TCAB and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) occur as contaminants in 3,4-dichloroaniline and its herbicidal derivatives, propanil, diuron, linuron and neburon. These contaminants are by-products of the commercial synthetic process (SUNDSTROM et al. 1978). Recently, some outbreaks of chloracne in groups of chemical workers have been attributed to TCAB and TCAOB (TAYLOR et al. 1979 and MORSE et al. 1979). In addition, several investigators have reported that the herbicides degrade in soil to produce TCAB and TCAOB (BARTHA et al. 1968). HSIA et al. (1977) has shown that TCAB is mutagenic, potentially carcinogenic, and quite toxic. POLAND and KENDE (1976) demonstrated the potent capacity of azobenzenes and azoxybenzenes to induce arylhydrocarbon-hydroxylase.

Some samples of TCAB-containing 3,4-dichloroaniline and propanil were tested for their porphyrinogenic potential in a primary tissue culture of chick embryo liver cells.

MATERIALS AND METHODS

Samples of 3,4-dichloroaniline and propanil were commercial products from different manufacturers and a gift from Dr. R.H. Hill, Centers for Disease Control, Atlanta, Georgia. He also determined the amount of TCAB in the different samples by high performance liquid chromatography (HILL et al. 1981). In the six samples used in this investigation the concentration of TCAB ranged from 4.2 µg/g in recristallized dichloroaniline to 1400 µg/g in the herbicide propanil.

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Chick embryo liver cell cultures were prepared according to the procedure of GRANICK et al. (1966) with some modifications (DEBETS et al. 1980). After 24 h incubation of the cell culture (37°C, 5% CO_2) the medium was replaced by 2 ml fresh medium and the TCAB-containing compounds, dissolved in ethanol, were administered. The concentration of dichloroaniline and propanil in the medium was 10 µg/mL. Accordingly, the final concentration of TCAB ranged from 0.04 to 14.0 ng/mL medium. The concentration of the solvent (ethanol) in the medium did not exceed 0.2%. This was shown not to be harmful to the cells or to evoke any accumulation of porphyrins. 24 h after addition of the TCAB containing compounds the intensity of porphyrin fluorescence of the liver cell cultures was determined semiquantitatively by fluorescence microscopy (scale ranging from 0-6) (DEBETS et al. 1980). Spectrophotometrically total porphyrin analyses in the liver cells according to HARMSEN and STRIK (1979) were made. Furthermore, the pattern of the different accumulated porphyrins was determined with the aid of thin layer chromatography followed by densitometric scanning of the developed porphyrin pattern according to the procedure described by STRIK and HARMSEN (1979).

RESULTS AND DISCUSSION

TCAB did show strong porphyrinogenic potential in chick embryo liver cell cultures. Porphyrins accumulated in the hepatocytes but not in the medium. Table 1 shows that samples of 3,4-dichloroaniline,containing a relatively low amount of TCAB, caused a slight porphyrin accumulation. Addition of propanil to the medium of chick embryo liver cell cultures caused a very high and a more or less maximal porphyrin accumulation in the hepatocytes. This latter porphyrin accumulation is about twice less than the porphyrin accumulation resulting from a treatment with 10 µg 2,4,5, 2',4',5'-hexachlorobiphenyl per mL medium. Hexachlorobiphenyl, a known porphyrinogenic compound in tissue culture of chick embryo liver cells (WIT 1972), was used as a positive control. So the porphyrinogenic potential of TCAB is about 500 greater than that of hexachlorobiphenyl.

TABLE 1. Porphyrin accumulation in chick embryo liver cell cultures induced by TCAB containing compounds.

Compound	TCAB contents (µg/g)	TCAB concentration in medium (ng/mL)	Microscopic fluorescence of cell cultures	Total porphyrins (ng/cell culture
3,4-dichloroaniline (techn.)	22.0	0.22	0	0.24
3,4-dichloroaniline (90%)	51	0.51	0	0.24
3,4-dichloroaniline (St.)	8.9	0.09	0	0.30
Propanil	1400	14.0	4	3.80
STAM-M-4 (Propanil)	1000	10.0	3	2.26
3,4-dichloroaniline (techn. recrystallized)	4.2	0.04	0	0.24
2,4,5,2',4',5' hexachlorobiphenyl (10 µg/mL medium)	-	-	5	6.30
Ethanol (solvent)	-	-	0	0.00

Inhibition of the enzyme uroporphyrinogen decarboxylase appears to be the primary lesion in the disturbance of the heme synthesis induced by TCAB in a chick embryo liver cell culture. This is indicated by the pattern of porphyrin accumulation in chick embryo liver cells. The porphyrins consist mainly of uro- and heptacarboxylic porphyrin after partial blocking of uroporphyrinogendecarboxylase and mainly of uroporphyrin after complete blocking of uroporphyrinogendecarboxylase by high amounts of TCAB (Figure 1).This type of porphyria is specifically caused by many other halogenated aromatics (STRIK et al. 1980).

In summary, TCAB showed a strong porphyrinogenic potential in liver cell cultures. Even a concentration as low as 0.1 ng TCAB/mL medium caused a significant accumulation of porphyrins, while a concentration of 10 ng/mL caused an excessive porphyrin accumulation, mainly consisting of uroporphyrin. The porphyrinogenic potential of TCAB is probably in the same range of 2,3,7,8,-tetrachlorodibenzodioxin. As far as the induction potential of δ-aminolevulinic acid synthase, rate limiting enzyme of the heme bio-

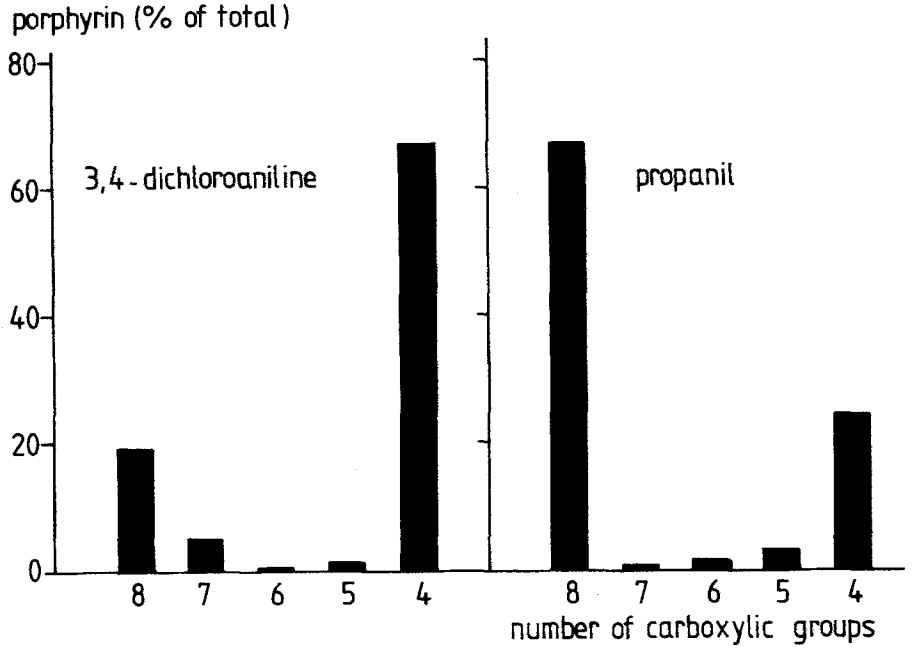

Figure 1. The relative distribution of porphyrins with different numbers of carboxylic groups accumulated in chick embryo liver cells, maintained in Williams E medium (plus 10% fetal calf serum) and exposed to 3,4-dichloroaniline (containing 4.2 µg/g TCAB) or propanil (containing 1400 µg/g TCAB). The accumulation of the intermediates is expressed as the percentage of total porphyrins formed. Non-exposed cultures contained only coproporphyrin. The intermediates are: 8,uroporphyrin; 7, heptacarboxylic porphyrin; 6, hexacarboxylic porphyrin; 5, pentacarboxylic porphyrin; 4, coproporphyrin.

synthetic pathway is concerned, POLAND and KENDE (1976) found an induction of δ-aminolevulinic acid synthase activity in livers of chick embryos after treatment with a single dose of 3.10^{-11} mol (∿ 10 ng) TCDD (in ovo). This active dose of TCDD is similar to the potent porphyrinogenic dose of TCAB in this study.

These results are in good agreement with the results obtained by HILL et al. (1981). They concluded, after investigating the same samples as used in this study, that the chloracnegenic potential of TCAB lies in the same range as that of TCDD. This is also true for the porphyrinogenic potential of TCAB.

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