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Porphyrinogenic Action of Fire Retardants

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Some environmental identified halogenated aromatics possess porphyrinogenic properties. Among them are fire retardants (STRIK et al., in prep.). The present study was carried out to determine the porphyrinogenic potential of some recently marketed fire retardants and Firemaster BP-6 in a primary tissue culture of chick embryo liver cells.

MATERIALS AND METHODS

All chemicals are commercial products and probably mixtures. Identity or purity was not verified. The following fire retardants were investigated:
decabromobiphenyl (DBB), decabromobiphenyloxide (DBBO), N,N'-ethylenebistetrabromophthalimide (TBPAED), octabromobiphenyloxide (OBBO), 1,2-bis(2,4,6-tribromophenoxy)-ethane (TBPE) and polybrominated biphenyl (Firemaster, BP-6).

Chick embryo liver cell cultures were prepared according to the procedure of GRANICK et al. (1975) with some modifications (DEBETS et al., in press). Some cultures were 20 h pretreated with β-naphthoflavone (β-NF, 3 µg/mL medium) to induce drug enzymes. After 24 h of culture the medium was replaced by 2 mL fresh medium and the fire retardants, either dissolved or suspended in dimethyl sulfoxide (DMSO), were administered. The concentration of DMSO in the medium was 1 µL/mL. At that concentration, the solvent did not harm the cells or caused any accumulation of porphyrins.

The intensity of porphyrin fluorescence of the liver cell cultures was determined semiquantitatively by fluorescence microscopy (scale ranging from 0-6), 24 h after addition of the fire retardants (DEBETS et al., in press).

RESULTS AND DISCUSSION

DBB and DBBO did not show any porphyrinogenic potential (Table 1). Oral doses of DBB did not cause porphyria in Japanese quail nor did administration in chick embryo liver cell culture (WIT 1972). Also decachlorobiphenyl was inactive in chick embryo liver cell culture (KAWANISHI et al. 1978). The nonporphyrinogenic effect of DBB and DBBO may be explained by their less planar molecular structure, which can be attributed to occupation of all *ortho* positions by

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Table 1. Porphyrinogenic activity of fire retardants in cultures of chick embryo liver cells.

Compound	Concentration (µg/mL medium)	Pretreatment (β-NF)	Microscopic fluorescence (score)
DBB[a]	10	-	0
	10	+	0
DBBO[a]	5	-	0
	5	+	0
TBPAED[b]	10	-	0
	10	+	trace
OBBO[a]	10	-	4-5
	10	+	4-5
TBPE[a/b]	2.5	-	0
	2.5	+	1
BP-6[a]	10	-	4
	10	+	4

[a]solution [b]suspension

bromine. Consistent with these results, 2,4,5,2',4',5'-hexachlorobiphenyl (HCB), 2,3,4,2',3',4'-HCB and 3,4,5,3',4',5'-HCB produced porphyria in chickens, whereas 2,3,6,2',3',6'-HCB and 2,4,6,2',4',6'-HCB did not (GOLDSTEIN et al. 1976). Of the chlorinated biphenyl isomers tested by KAWANISHI et al. (1978) in tissue culture of chick embryo liver cells 2,6,2',6'-tetrachlorobiphenyl was not porphyrinogenic.
Moreover, DBB and DBBO are very resistant to metabolic degradation. There have been found no hydroxylated metabolites of decachlorobiphenyl in the rat (SUNDSTRÖM et al. 1976, GOTO et al. 1975). This can be another explanation for the incapability of these compounds to induce porphyria, since there is growing evidence that the porphyrinogenic effect of halogenated aromatic hydrocarbons is mediated through reactive metabolic intermediates (DEBETS & STRIK 1979, DEBETS et al. in press).

It is likely that the absorption and diffusion of different halogenated biphenyl congeners are greatly dependent on the stereochemistry of the individual isomers. The steric orientation of the voluminous halogen substituents may prevent interaction with protein synthesis regulatory mechanism of the cell nucleus (WIT 1972, KAWANISHI et al. 1978).

TBPAED and TBPE are slightly porphyrinogenic only after pretreatment with β-NF. Addition of β-NF alone causes no accumulation of porphyrins in tissue culture. Pretreatment of chick embryo liver cell culture with β-NF causes induction of cytochrome P-450 and P-448 (ALTHAUS et al. 1979) and δ-aminolevulinic acid synthase (δ-ALAS) (2-fold increase; DEBETS, unpublished results). TBPAED and TBPE might be not capable of inducing drug metabolism. The pre-induction of cytochrome(s) P-450 by β-NF may have led to a faster metabolic conversion

of these polyhalogenated biphenyls. Consequently, their porphyrinogenic action may be earlier manifested. Merely the slight increase in the activity of δ-ALAS is not responsible for an expression of the porphyrinogenic potential (STRIK 1973). The poor solubility of TBPE and TBPAED might be another factor responsible for their low activity.

OBBO and BP-6 are, strongly porphyrinogenic, even without pre-treatment with β-NF (Table 1). WIT (1972) reported the porphyrinogenic activity of BP-6 in chick embryo liver cell culture. BP-6 is porphyrinogenic also in animals, e.g. Japanese quail (STRIK 1973). It remains to be investigated if the porphyrinogenic effect of OBBO can be mainly attributed, either to the presence of highly active impurities like dibenzodioxins and lower brominated congeners, or to the OBBO isomers themselves.

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