Further Toxicologic Studies With Commercial And Candidate Flame Retardant Chemicals. Part II.

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Whereas much research has been conducted on the possible hazards of combustion products of flame retardant fabrics (AUTIAN, 1970) relatively little has been reported on the toxicology of intact flame retardant chemicals. The toxicity to fish of flame retardant chemicals added to water or released from commercially treated fabrics immersed in water was reported* (GUTENMANN and LISK, 1975). The flame retardant TDBPP (tris(2,3-dibromopropyl) phosphate), used on virtually all children's polyester sleepwear was reported to be released when the fabric was in contact with water* (GUTENMANN and LISK, 1975). When applied to the shaven skin of a rat, TDBPP penetrated and a metabolite, 2,3-dibromopropanol (DBP) was excreted in the animals urine freely and as a conjugate. TDBPP was hydrolyzed in vitro to DBP by the 10,000xg supernatant fraction of rat liver (ST. JOHN et al., 1976). However, humans wearing TDBPP-treated flame retardant polyester sleepwear for 7 nights did not excrete detectable quantities of DBP in their urine (ST. JOHN, et al., 1976).

In the work reported, determination was made of the extent to which various commercial and candidate flame retardant chemicals (1) inhibited Torpedo electric organ acetylcholinesterase (AChE) activity, (2) inhibited binding of acetylcholine (ACh) to its Torpedo electric organ receptor, (3) inhibited the southern armyworm (Spodoptera eridania Cramer) midgut microsomal p-chloro-N-methylaniline N-demethylase enzyme system, (4) synergised insecticide toxicity, and (5) killed fish, houseflies and armyworms.

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* Reported in Part I of this study

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Table 1. Flame retardants studied and their commercial uses.

Flame retardant	Use
Antiblaze 19 (a mixture of cyclic phosphonates)	textiles, plastics
Antiblaze 78 (a mixture of monomeric chloroethyl phosphonates and high boiling phosphonates)	polyurethane foams
Fyrol CEF (tris (beta chloroethyl phosphate)	textiles
Fyrol FR-2 (tris (dichloropropyl) phosphate)	polyurethane foams
Fyrol 76 (an oligomeric vinyl phosphonate)	cellulosic textiles
Phosflex 179-C (tri (orthocresol) phosphate)	vinyl, cellulosic plastics
Phosflex 300 (mixed triaryl phosphate esters containing halogen)	vinyl resins
Phosflex 400 (mixed triaryl phosphate esters containing halogen)	vinyl resins
Pyrovatex CP (N-methylol dimethyl phosphonopropionamide)	cellulosic textiles
TDBPP (tris (2,3-dibromopropyl) phosphate)	polyester textiles, polyurethane and polystyrene foams
THPOH (tetrakis(hydroxymethyl) phosphonium hydroxide)	cellulosic fabrics

EXPERIMENTAL

The flame retardant compounds studied and their uses are listed in Table 1. The toxicity of flame retardant chemicals to goldfish (Carassius auratus) was investigated by dissolving the compound in water (or in 5 ml of acetone) and introducing the solution into 20 liters of water (electrical conductivity, 290 micromhos/cm., temperature 20° C) in a tank containing 6 goldfish, each about 3 inches long. Toxic symptoms and fish mortality were then noted as a function of time.

The extent to which the various flame retardants inhibited (1) the enzyme AChE and (2) binding of ACh to its receptor was determined. The source of AChE and ACh-receptor was the membranes (pellet of 17,000 rpm, 60 min) of the electric organ of the electric ray, Torpedo ocellata. They were suspended in buffered saline (0.2 M NaCl, 5 mM Na_2HPO_4, pH 7.4) and contained on the average 1 nmole of ACh-receptor sites and 0.5 nmole of AChE catalytic sites per ml, representing 1 g tissue. Each 1 ml of membrane preparation was incubated with the flame retardant at 23° C for 1 h, then AChE activity measured in 0.1 ml (after dilution 500x with buffered saline) using the method of ELLMAN et al. (1961). Binding of (^{3}H)ACh (sp. act. 49.5 Ci/mole, from New England Nuclear) to its receptor was determined after inhibition of its AChE by incubation with 0.1 mM DFP (diisopropyl phosphorofluoridate) for 1 h at 23° C, a treatment which does not affect ACh-receptor (AChR) binding (ELDEFRAWI et al., 1971a). For the binding, equilibrium dialysis was used at 4° C for 16 h in the presence of 1 µM (^{3}H)ACh and 0.01 mM DFP, as previously described (ELDEFRAWI, et al., 1971b; EDELSTEIN et al., 1975). Experiments were run in triplicate.

The inhibitory effect of flame retardants to the microsomal p-chloro N-methylaniline N-demethylase enzyme system was measured. The incubation mixtures consisted of 50 mM tris HCl, pH 7.8, 27.8 mM KCl, 51 µM NADP, 2.4 mM glucose-6-phosphate (G-6-P), 1.6 units of G-6-P dehydrogenase, 0.6 mM p-chloraniline and 1 mg microsomal protein in a total volume of 5.0 ml. The microsomal mixed function oxidase (MFO) enzyme was obtained from midguts of sixth instar larvae of the southern armyworm Spodoptera eridania Cramer and the enzyme assays were performed as described by BRATTSTEN and WILKINSON (1973).

The toxicity of flame retardant compounds to houseflies was determined by the method of PLAPP and CASIDA (1970). The flame retardant was incorporated into a diet consisting of 0.5 g each of sugar and dry milk and fed to 2 to 3 day old, female houseflies (Musca domestica L, standard insecticide susceptible World Health Organization strain).

RESULTS AND DISCUSSION

Table 2 lists the solubilities in water, oral LD_{50} values for rats and observed toxicity to goldfish of the compounds studied. Fyrol FR-2, Phosflex 300 and 400, TDBPP and THPOH were notably toxic to goldfish. Toxicity was most conspicuously associated with sluggish and disoriented swimming prior to death.

Inhibition by the flame retardants of membrane-bound AChE and AChR binding is listed in Table 3. Several compounds inhibited Torpedo AChE activity, but none was as strong as the potent AChE inhibitor, DFP. Also none of the compounds interferred with binding of ACh to its receptor, which is neuromuscular nicotinic in its pharmacology. At a similar mM concentration, a receptor activator or inhibitor (e.g. nicotine and curare, respectively) would inhibit most or all of the ACh binding to its receptor.

Table 2. Water solubility, LD_{50} (mg/kg, oral-rats) and toxicity to gold fish of flame retardants.

Flame retardant (fr)	Water Solubility ppm	LD_{50}	No.* of dead fish after time and fr conc. shown		
			ppm	hours	no.
Antiblaze 19	very soluble		15	168	0
Antiblaze 78			15	168	0
Fyrol CEF	7000	1230	5	168	0
Fyrol FR-2	100	2830	1	168	0
			5	24	6
Fyrol 76	<1000	937	15	168	0
Phosflex 179-C	3	3000	5	168	0
Phosflex 300	7000		1	168	5
			5	5	6
Phosflex 400	5000		5	168	3
Pyrovatex CP	soluble	>10,000	15	168	0
TDBPP	1.6	5240	1	120	6
THPOH	soluble	232	1	408	5

* A total of 6 fish were exposed.

Table 3. The effect of flame retardants on membrane-bound AChE and AChR of Torpedo electroplax.

Flame retardant (10^{-3}M)	% of control	
	AChE	AChR
Antiblaze 19	103.4	98.8
Antiblaze 78	114.5	101.4
Fyrol CEF	73.7	107.2
Fyrol FR-2	79.1	93.9
Fyrol 76	114.8	103.2
Phosflex 179-C	82.2	109.8
Phosflex 300	75.1	110.1
Phosflex 400	92.9	107.0
Pyrovatex CP	80.8	94.2
TDBPP	81.1	99.9
THPOH*	79.5	76.7
DFP (10^{-4}M)	0.3	99.8

* This compound caused visible protein aggregation when added to the membrane preparation.

Table 4 lists the depression (% of control) of the microsomal p-chloro N-methylaniline N-demethylase enzyme system of the southern armyworm when 5 mg of various of the compounds were included in the incubation mixture. Also shown is the percent mortality for houseflies when various of the flame retardant compounds were mixed alone at 1% (w/w) with their sugar and dry milk diet or when 5% piperonyl butoxide was also included in their diet. As is evident, THPOH totally inhibited the armyworm MFO enzyme while Fyrol 76 and Pyrovatex CP showed little effect. None of the compounds studied can be considered potent inhibitors of the enzyme, however, when compared to the commercially used insecticide synergist, piperonyl butoxide which reduces enzyme activity by 50% at a concentration about 2000 times lower.

Table 4. Depression of southern armyworm microsomal p-chloro N-methylaniline N-demethylase and housefly mortality in the presence of synergised and unsynergised flame retardants.

Flame retardant (fr)	Armyworm MFO enzyme depression %	Housefly mortality (%) after 72 hours	
		1% fr	1% fr + 5% pb[1,2]
Fyrol CEF	53	0	70
Fyrol FR-2	40	5	75
Fyrol 76	98		
Phosflex 179-C		0	90
Phosflex 300		5	95
Phosflex 400		0	70
Pyrovatex CP	88		
TDBPP	57		
THPOH	2		

1 The insecticide synergist piperonyl butoxide
2 5% piperonyl butoxide alone in the diet caused 5% housefly mortality in 72 hours

None of the compounds in Table 4 caused appreciable housefly mortality after 24 hours and only Fyrol FR-2 and Phosflex 300 caused 5% mortality after 72 hours when present alone at 1% in their diet. The inclusion of 5% piperonyl butoxide, however greatly increased mortality as shown. When the diet included 0.5% TDBPP and 2.5% piperonyl butoxide, 90% mortality was observed in 96 hours. TDBPP must therefore be considered only weakly insecticidal. The addition of 0.5% TDBPP to a diet containing 0.1% of the insecticide, carbaryl (N-methyl-1-naphthylcarbamate)

increased housefly mortality from 40% for carbaryl alone to 100% in 24 hours. Fyrol CEF, Fyrol FR-2, Fyrol 76, Pyrovatex CP, TDBPP and THPOH were not toxic to houseflies when applied topically. TDBPP could not be synergised by piperonyl butoxide by topical application to houseflies. None of the compounds showed toxicity to southern armyworms when they were allowed to feed for 24 hours on kidney bean foliage to which a 0.1 or 1.0% solution of the TDBPP, THPOH, Fyrol 76 or Pyrovatex CP had been applied. None of these latter four compounds reduced the toxicity of the insecticide malathion (0,0-dimethyl S-bis(carboethoxy)ethyl phosphorodithioate) to armyworms when mixed with it prior to application to the foliage, thus indicating that they did not inhibit the enzyme in vivo which converts malathion to malaoxon.

Of the compounds examined Fyrol FR-2, Phosflex 300 and 400, TDBPP and THPOH were notably toxic to goldfish and showed moderate cholinesterase inhibition. As noted earlier TDBPP is used almost exclusively on children's polyester sleepwear. Its demonstrated migration out of fabrics even after repeated laundering in quantities sufficient to kill goldfish and its ability to penetrate rat skin with urinary excretion of its hydrolysis product 2,3-dibromopropanol (ST. JOHN et al., 1976) is cause for concern. TDBPP would be considered moderately toxic based on its LD_{50} (Table 2). However the compound (or perhaps in reality its metabolite demonstrated in Part I of this study) has been shown to be mutagenic by the test of AMES (1971). Based on total bromine analysis, residues of TDBPP accumulate in tissues of rats fed the compound (KERST, 1974). Indeed TORMALA (1970) warns against the use of anticholinesterase lipophilic organophosphates as plasticizers in synthetic films intended for food packaging. Whether the compound released from treated fabrics into water would pose a hazard to laundry workers has not been studied. Appreciable quantities of TDBPP might be released into foods in the event that treated fabric was used in place of cheesecloth as a "fruit bag" from which hot juices are expressed during preparation of jellies for instance. Considerable quantities of TDBPP would expectedly be removed and contact the skin if organic solvents were applied to portions of treated fabric prior to their use for paint or varnish removal.

From the data gathered so far TDBPP appears to be mainly a potent fish poison. Whether its mode of toxic action is solely inhibition of cholinesterase or mixed function oxidases is unknown. Further studies are necessary to determine possible latent toxic effects of TDBPP and other flame retandant chemicals on a range of biological organisms, most importantly, humans.

SUMMARY

A number of commercial and candidate flame retardants were studied with regard to their toxicity to goldfish, inhibition of cholinesterase, inhibition of acetyl choline binding to its receptor and insecticidal properties. Several of the flame retardants were notably toxic to fish. Some of the compounds showed modest inhibition of cholinesterase and/or microsomal oxidases, but none inhibited acetyl choline receptor binding. Whereas several of the flame retardants showed little or no insecticidal properties when added alone to a housefly diet, piperonyl butoxide greatly synergised their toxicity to houseflies.

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