Tris(2,3-Dibromopropyl) Phosphate: Mutagenicity of a Widely Used Flame Retardant

Abstract. *Tris(2,3-dibromopropyl) phosphate, a widely used flame-retardant additive for textiles, is mutagenic to histidine-requiring strains of* Salmonella typhimurium. *Extracts of fabrics treated with this compound are also capable of inducing mutations in these bacterial strains.*

The most widely used flame-retardant additive for children's sleepwear is tris(2,3-dibromopropyl) phosphate [tris-BP; $(Br\text{-}CH_2\text{-}CH(Br)\text{-}CH_2\text{-}O)_3P{=}O$] (*1*). Its use is particularly intensive in sleepwear from newborn size to size 6X, although larger sizes of flame-resistant children's sleepwear may also contain tris-BP. Although investigation of the eye and skin irritation potential and acute and subacute toxicity of tris-BP have been reported (*2*), no data are available on its long-term toxicity, carcinogenicity, or mutagenicity.

Ames and his co-workers have presented evidence that the ability of a chemical to induce mutations in bacteria in the presence of liver microsomes correlates well, although not perfectly, with carcinogenic potential (*3*). In this report, we show that tris-BP is mutagenic to bacteria and that this mutagenic activity is retained when this chemical is used to treat fabric.

For the detection and classification of mutagens, the *Salmonella typhimurium* assay for mutagenicity and the bacterial strains developed by Ames and his associates were used (*4*). This procedure is based on the mutagenic conversions of indicator strains from histidine dependence to histidine independence. Some of the strains (for example, TA1535) revert to histidine independence as a result of base-pair substitution mutations, while others (for example, TA1537 and TA1538) respond only to frameshift mutagens. In this study we also used the recently constructed strain TA100, which has an enhanced susceptibility to certain mutagens as a result of the presence of a plasmid (*4, 5*). For metabolic activation, hepatic microsomes (S-9 fraction) derived from normal Sprague-Dawley rats as well as from animals induced

Table 1. Mutagenicity of flame retardants and impurities in *Salmonella*. "Amount" indicates the volume of undiluted test compound per plate. All tests were performed as in Fig. 1. Abbreviations: S9, S-9 fraction derived from normal animals; S9A, S-9 derived from Aroclor-induced animals; LV, low in volatiles; and HV, high in volatiles.

Experiment	Substance	Amount (μl)	Mutants per plate								
			TA100			TA1535			TA1538		
			−S9	+S9	+S9A	−S9	+S9	+S9A	−S9	+S9	+S9A
1	Tris-BP, prep 1 (LV)	0	188	196	179	15	11	20	14	12	16
		0.01	280	680	1060	14	81	231	16	16	21
		0.1	330	760	2450	26	183	1120	12	16	18
		1.0	450	960	2940	100	243	1050	12	17	17
		10	1060	1320	3220	1120	370	1020	9	13	17
2	Tris-BP, purified	0	190	178	201	23	16	19			
		0.01				22	28	510			
		0.1				32		1020			
		1.0	375	940	3010	174	320	1050			
		10				1184	398	910			
3	Tris-BP, prep 2 (HV)	1				41	230	1160	14	17	19
	prep 3 (LV)	1				95	263	880			
	prep 4 (LV)	1				132	165	810			
4	Tris-BP, purified	0.1				26	217	1160			
		0.1					32*	41*			
		0.1					83†	61†			
5	1,2,3-Tribromopropane	0				3		7	3		
		0.01				11		110	5		7
		0.1				63		352	4		5
		1.0				580		159	0		1
		10				70		7			0
6	2,3-Dibromopropanol	0				12			3		
		0.01				15		33	5		10
		0.1				24		120	6		9
		1				223		860	4		7
		10				10		28	4		0
7	1,2-Dibromo-3-chloropropane	0				19		56			
		0.01				66		106	10		15
		0.1				165		28			
		0.5				17		23	10		5
		1				18					
8	Tris (1-chloromethyl-2-chloroethyl) phosphate	0	89	109	91	12	11	10	9	7	9
		1	103	79	80	14	12	14	7	11	9
		10	90	88	83	10	9	12	10	8	10
	Tris (2-chloroethyl) phosphate	1	81	98	75	13	14	11	11	13	9
		10	97	101	81	17	11	9	7	8	6

*S-9 fraction without added nicotinamide adenine dinucleotide phosphate (NADP) or glucose 6-phosphate. †S-9 fraction heated at 60°C for 15 minutes.

with Aroclor 1254 (a commercial mixture of chlorinated biphenyls) were prepared (*4*).

The mutagenicity of tris-BP for *Salmonella typhimurium* TA1535 and TA100, but not TA1538, was readily demonstrable (Fig. 1 and Table 1, experiments 1 to 4). Tris-BP also failed to induce mutations in strain TA1537 (data not shown). These results indicate that tris-BP induces mutations of the base-pair substitution type. Although at higher concentrations [>1 μl per plate (density, 2.27 g/cm^3)] tris-BP behaves as a direct-acting mutagen not requiring activation by hepatic microsomes, much lower concentrations (0.01 μl per plate) of this agent demonstrated significant genetic activity only when the microsomal preparation was present (Fig. 1 and Table 1, experiments 1 and 2). When the S-9 fraction was heated or cofactors were omitted from the reaction mixture, activation of tris-BP was greatly reduced (Table 1, experiment 4). This demonstrates that activation by the S-9 is due to enzymatic activity.

The complex dose-response curves obtained with tris-BP (Fig. 1) imply that there are at least two different active mutagens in the reaction mixtures. These may be either two different metabolites of tris-BP or one metabolite and tris-BP itself. This observation cannot be considered surprising in view of the fact that the tris-BP molecule has two different structural properties that are shared by other classes of mutagens; that is, it is a trialkyl phosphate and it is also an alkyl bromide. The conclusion that at least two active mutagenic species are involved is reinforced by the finding that at high concentrations of tris-BP (10 μl per plate) the mutagenic response in the absence of microsomes is frequently greater than that seen in the presence of microsomes from uninduced animals. It is possible that a major product formed by uninduced liver enzymes is 2,3-dibromopropanol, which exhibits a lower level of mutagenicity than tris-BP (Table 1, experiment 6). It has been shown that 2,3-dibromopropanol is a metabolite of tris-BP in rats (*6*).

It was repeatedly observed that the use of microsomal preparations derived from animals first treated with Aroclor 1254 resulted in a different dose-response curve than did the use of preparations obtained from untreated animals. These findings suggest that the enzymic activities present in the two types of preparations differ both qualitatively and quantitatively.

Commercial preparations of tris-BP can be obtained in two grades: HV (high in volatiles) and LV (low in volatiles). A typical LV sample is reported to contain 0.05 percent 1,2-dibromo-3-chloropropane, 0.05 percent 1,2,3-tribromopropane, and 0.20 percent 2,3-dibromopropanol (*2*). On a quantitative basis, no significant difference in mutagenic activity was observed among nine different commercial samples, including HV and LV materials, obtained from five different suppliers (Table 1, experiments 1 and 3). In addition, a highly purified sample of tris-BP, containing 0.029 percent 1,2,3-tribromopropane and less than 0.002 percent each of 1,2-dibromo-3-chloropropane and 2,3-dibromopropanol, had approximately the same mutagenic activity as the commercial samples (Table 1, experiments 1 and 2). Although each of the three listed contaminants displays some mutagenic activity (Table 1, experiments 5 to 7) (*7*), this activity is not sufficient to account for the mutagenicity of tris-BP when the level of these chemicals in tris-BP is taken into account. It is thus highly unlikely that the mutagenicity of tris-BP is due to the presence of an impurity.

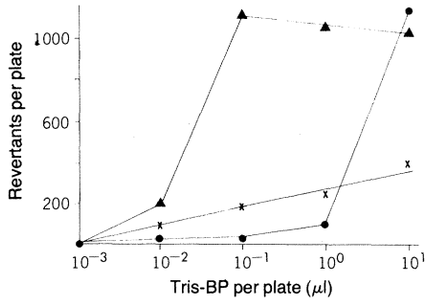

Fig. 1. Mutagenicity of tris(2,3-dibromopropyl) phosphate for *Salmonella typhimurium* TA1535. Tris-BP was diluted 1 : 10 into dimethyl sulfoxide. Bacteria, together with diluted test agent, were incorporated into the agar overlay. Some of the plates were supplemented with the S-9 (microsomal) fraction derived from rat liver (400 μg of protein per plate). (•) No enzyme added; (×) S-9 fraction derived from normal liver; and (▲) S-9 fraction from Aroclor 1254–induced rat liver. The plates were incubated in the dark (*14*) for 46 hours and revertants to histidine independence were enumerated. The quantity expressed on the abscissa is volume of undiluted tris-BP.

Four pairs of fabric samples, matched in all respects except that one sample in each pair had been treated with tris-BP, were extracted with dimethyl sulfoxide to determine whether the mutagenic activity was retained by the fabric. Mutagenic activity that was enhanced by the presence of microsomes from Aroclor-induced rats was extractable from tris-BP–treated fabrics by dimethyl sulfoxide (Table 2). Even after three cycles of laundering with detergent, some mutagenic activity was still demonstrable in the extracts. Water extracts of tris-BP–treated fabrics appeared to increase the number of revertants of strain TA100, but the effect was generally small (less than two-fold increase over background) and was not consistently observed.

Tris(1-chloromethyl-2-chloroethyl) phosphate (*8*) and tris(2-chloroethyl) phosphate, two halogenated trialkyl phosphates closely related to tris-BP chemically and used as flame retardants in certain plastics, were devoid of mutagenic potential when assayed under the conditions employed in this study (Table 1, experiment 8).

Application of tris-BP to the shaved skin of a rat resulted in the appearance of free and conjugated 2,3-dibromopropanol in the urine (*6*). Humans who had been sensitized to tris-BP exhibited skin reactions when exposed to tris-BP–treated fabric (9). These results suggest

Table 2. Mutagenicity for *Salmonella typhimurium* TA1535 of dimethyl sulfoxide extracts of matched tris-BP–treated and untreated fabrics. One-gram quantities of fabric were extracted for 18 hours with 5 ml of dimethyl sulfoxide at 37°C with shaking. The dimethyl sulfoxide extracts were then sterilized by filtration and tested for mutagenicity in *Salmonella typhimurium* TA1535 (*4*). Abbreviations are as in Table 1.

Sample	Tris-BP	Description of fabric	Microliters per plate	Colonies per plate	
				−S9	+S9A
D	−	100 percent polyester, circular knit, white	10	17	14
C	+	Same as D except tris-BP–treated	10	5	685
		Background mutants		7	10
P	−	100 percent polyester, broadcloth, white	100	7	11
S	+	Same as P except tris-BP–treated	100	30	445
		Background mutants		9	
Q	−	100 percent polyester flanellette, white	200	5	7
R	+	Same as Q except tris-BP–treated	200	30	368
		Background mutants		9	
11	−	100 percent polyester twill, dyed green	100	6	35
10	+	Same as 11 except tris-BP–treated	100	110	430
		Background mutants		3	5

that some amount of tris-BP can be readily released from fabric and may be absorbed through the skin, but further studies are needed to confirm this.

While positive results in mutagenicity tests in bacteria are usually reliable indicators of carcinogenic potential in mammals, some mutagens may not be carcinogens (*3, 10*). Thus, our results do not conclusively demonstrate that tris-BP is a cancer-causing agent. Another concern raised by the results is that tris-BP may cause heritable mutations in humans. Mutagenicity in bacteria does not, in itself, imply that a chemical poses a genetic risk to humans because the chemical might not reach the germ cells of exposed humans in an active form or might not be mutagenic to eukaryotic cells. The latter possibility is excluded for tris-BP by observations indicating that this chemical induces heritable mutations (sex-linked recessive lethals) in *Drosophila melanogaster* (*11*) as well as unscheduled DNA synthesis (*12*) and repairable breaks in DNA (*13*) in human cells in culture. A complete assessment of the mutagenic hazard associated with the use of tris-BP in children's sleepwear requires additional data on the possible oral and dermal absorption of the chemical from fabric as well as data on whether or not tris-BP or its active metabolites, once absorbed, can reach germinal tissue. More definitive evaluation of the carcinogenic potential of tris-BP must await the completion of tumor-induction studies in mammals.

MICHAEL J. PRIVAL*
Office of Toxic Substances WH-557, Environmental Protection Agency, Washington, D.C. 20460
ELENA C. MCCOY
BEZALEL GUTTER
HERBERT S. ROSENKRANZ
Department of Microbiology, New York Medical College, Valhalla 10595

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* Present address, Genetic Toxicology Branch, HFF-156, U.S. Food and Drug Administration, Washington, D.C. 20204.

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Activation of the Nigrostriatal Dopaminergic Pathway by Injection of Cholera Enterotoxin into the Substantia Nigra

Abstract. *Twenty-four hours after unilateral injection of cholera enterotoxin into the rat substantia nigra there is an increase, in the striatum on the injected side, of basal adenylate cyclase activity, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid. Moreover, there is an increase of motor activity, and rats tend to circle contralateral to the side of the injection. Injection of cholera enterotoxin into brain nuclei may be a useful procedure for pharmacologically activating selected neuronal systems of brain and for studying the pharmacology of drugs that are suspected of interacting with these systems.*

Cholera enterotoxin (choleragen) binds to cell surface receptor glycolipids and stimulates membrane-bound adenylate cylase (*1*). The stimulation by choleragen is long lasting. We found that selected neuronal systems of rat brain were biochemically activated for prolonged periods if choleragen was injected into specific nuclei of brain. Activation may be the consequence of stimulating adenylate cyclase systems.

The dopaminergic neurons of the striatum originate in the substantia nigra (*2*), and destruction of the substantia nigra leads to a loss of dopaminergic neurons in the striatum (*3*). In contrast, we found that injection of choleragen into the substantia nigra produced biochemical changes in the striatum that are consistent with the hypothesis that dopaminergic neurons are activated. Biochemical activation of the striatum was manifested by an increase of basal adenylate cyclase activity, an elevation of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid [homovanillic acid (HVA)] concentrations, and an increase of motor activity. These changes lasted for more than 24 hours. Local injection of choleragen into nuclei of brain may be a useful procedure for pharmacologically activating specific neuronal pathways and for evaluating the actions of drugs that are thought to alter these pathways.

Male Sprague-Dawley rats (150 to 180 g), anesthetized with chloral hydrate, were stereotaxically injected with 1 μg of choleragen (Schwarz/Mann, Orangeburg, N.Y.) in 1 μl or with vehicle unilaterally into the substantia nigra. The stereotaxic coordinates, according to Konig

Table 1. Basal and dopamine-stimulated adenylate cyclase activity of the rat striatum after injection of choleragen into the substantia nigra. Choleragen (1 μg) was injected unilaterally into the substantia nigra 24 hours before adenylate cyclase was assayed in the striata from the injected and contralateral sides of the brain. Enzyme activity (nanomoles of cyclic AMP per milligram of protein per minute) was measured in the absence (basal activity) and presence (dopamine-stimulated activity) of 0.1 m*M* dopamine. Values are means ± standard error of the mean (S.E.M.). Incubation was for 3 minutes at 30°C. A paired *t*-test was used to compare basal and dopamine-stimulated adenylate cyclase activity from the same side of the brain and activity in the striatum from the injected and contralateral sides of the brain.

Treatment	Enzyme activity			
	Injected side		Contralateral side	
	Basal	Dopamine-stimulated	Basal	Dopamine-stimulated
Toxin ($N = 10$)	0.13 ± 0.02*	0.18 ± 0.02*†	0.071 ± 0.005	0.11 ± 0.01‡
Vehicle ($N = 5$)	0.065 ± 0.006	0.11 ± 0.01§	0.068 ± 0.003	0.11 ± 0.01§

*$P < .05$ compared to contralateral. †$P < .05$ compared to basal. ‡$P < .001$ compared to basal. §$P < .01$ compared to basal.