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Mutagenicity of bis- and mono-(2,3-dibromopropyl)phosphate, and their salts used as flame retardants, in the Salmonella/microsome system

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Summary

The mutagenicity of pure synthesized samples of bis(2,3-dibromopropyl)phosphate (bis-BP) and mono(2,3-dibromopropyl)phosphate (mono-BP) against *Salmonella typhimurium* TA100 was examined in relation to microsomal activation of tris(2,3-dibromopropyl)phosphate (tris-BP). Both mono- and bis-BPs were weak direct mutagens. Their mutagenicities increased with S9 mix, but the rates were much less than that of tris-BP.

The magnesium and ammonium salts of mono- and bis-BPs were also prepared and their mutagenicities were examined with S9 mix in relation to 2 commercial flame retardants (our abbreviations: DB-1 and DB-2). In both mono- and bis-BP series, an apparent increase of mutagenicity was observed in the order: ammonium salt > magnesium salt > free acid. On the other hand, mono-BP and its salts are usually more active than the corresponding bis-BPs independently of the kind of cation.

DB-1 (DB-2), however, is more potent than the magnesium (ammonium) salts of mono- and bis-BPs, the constituents in DB-1 (DB-2). No synergistic effect between mono-BP salts and bis-BP salts was observed. The different unknown mutagenic compounds in DB-1 and DB-2 are suggested.

Tris(2,3-dibromopropyl)phosphate (tris-BP) induces tumors in the kidney of mice and rats [6,11,17], and it is mutagenic for *Salmonella typhimurium* [1,4,6,10,13]. Though tris-BP is a direct-acting mutagen at high concentrations, its mutagenicity markedly increases in the presence of liver microsomes. 2,3-Dibromopropanol is a

metabolite of tris-BP in rats [15] and in humans [2], but is less mutagenic than tris-BP [1,4,6,10,13]. Lynn et al. (1980) reported another metabolite, bis(2,3-dibromopropyl)phosphate (bis-BP), which was detected in rat urine by high-performance liquid chromatography [8]. Therefore, mono(2,3-dibromopropyl)phosphate (mono-BP) should also be considered as one of the metabolites. To elucidate the possible pathways leading to mutagenic products from tris-BP biotransformation, it is necessary to test the mutagenicity of the pure samples of mono-BP and bis-BP.

On the other hand, magnesium and ammonium salts of the mixture of bis- and mono-BPs are not necessarily well known to be used as flame-retardant additives [3,12]. In the previous paper [10], we reported that they were also mutagenic in the presence of rat-liver microsomes, where, however, we called them magnesium bis(2,3-dibromopropyl)phosphate (DB-1) and bis(2,3-dibromopropyl)phosphoamide (DB-2). The magnesium salt of the mixture of bis- and mono-BPs, DB-1, was recently found to be an animal carcinogen by the Department of Toxicology, National Institute of Hygienic Sciences, Japan [16], and DB-1 and DB-2 were banned for use in textile products by the Japanese Government in 1981 [7].

In this paper, synthesized pure samples of mono-BP, bis-BP, and their magnesium and ammonium salts were tested for mutagenicity against *S. typhimurium* in relation to microsomal activation of tris-BP and to the mutagenicity of DB-1 and DB-2.

Materials and methods

Chemicals

Test compounds are listed in Table 1. Mono-BP and bis-BP were synthesized in

TABLE 1

COMPOUNDS TESTED AND THEIR ABBREVIATIONS

(i) Synthesized compounds

Mono-BP: $BrCH_2CHBrCH_2O-P(=O)(OH)(OH)$

Bis-BP: $(BrCH_2CHBrCH_2O)_2P(=O)-OH$

Mono-BP-Mg: $BrCH_2CHBrCH_2O-P(=O)(O^-)(O^-)\,Mg^{2+}$

Bis-BP-Mg $(BrCH_2CHBrCH_2O)_2P(=O)-O^-\,\frac{1}{2}Mg^{2+}$

Mono-BP-NH_4 $BrCH_2CHBrCH_2O-P(=O)(O^-)(O^-)\,2NH_4^+$

Bis-BP-NH_4 $(BrCH_2CHBrCH_2O)_2P(=O)-O^-\,NH_4^+$

(ii) Commercial flame retardants

Tris-BP: $(BrCH_2CHBrCH_2O)_3P=O$

DB-1 [a]: $P_2O_5 + 3\ BrCH_2CHBrCH_2OH \rightarrow [\ ] \xrightarrow{Mg(OH)_2}$ DB-1

DB-2 [a]: $P_2O_5 + 3\ BrCH_2CHBrCH_2OH \rightarrow [\ ] \xrightarrow{NH_4OH}$ DB-2

[a] DB-1 and DB-2 were prepared according to the schemes shown in the Table Their trade names are NON-NENs (Marubishi Yuka Kogyo Co Ltd.)

our laboratory. Their purities and structures were confirmed by elemental analysis, gas chromatography, IR, NMR and mass spectrometries. The details of analysis will be reported in a separate paper [9]. Gas-chromatographic analysis indicates that bis-BP has a purity of 94.2% and contains 1.5% of mono-BP and trace amounts of unknown compounds, and that mono-BP is almost pure.

Magnesium and ammonium salts of mono- and bis-BPs were prepared as follows. To 0.005 mole of mono- or bis-BP were added 40 ml of water and a slight excess of magnesium hydroxide or ammonia water, and the mixture was stirred until it became clear; then the solution was freeze-dried.

The contents of mono-BP and bis-BP salts in the flame-retardant additives, DB-1 and DB-2, were also determined by gas chromatography [9]. DB-1 (colorless powder) contained the corresponding magnesium salts to 26.8% of bis-BP and 48.0% of mono-BP, and DB-2 (brown viscous aqueous solution) contained the corresponding ammonium salts to 12.2% of bis-BP and 10.1% of mono-BP.

Mutation assay

Only strain TA100 of *Salmonella typhimurium* was used, because the chemicals were already known to induce base-pair substitution mutation. Bis-BP and its salts were dissolved in dimethyl sulfoxide, and mono-BP and its salts were dissolved in water. The assay was carried out as described in the previous paper [10].

Results and discussion

Table 2 shows the results of the tests for mono-BP, bis-BP and tris-BP. Weak direct mutagenicities were observed in both mono-BP and bis-BP. In the presence of S9 mix, a marked increase of mutagenicity was found for mono-BP, but it was slight for bis-BP. Tris-BP, however, was more than 25 times as active as mono- and bis-BPs in the presence of S9 mix. These results show that mono- and bis-BPs do not play a main role in the metabolic activation of tris-BP, and support the consideration by Søderlund et al. (1979) [14] who suggested that ester hydrolysis is not involved in the activation of tris-BP to mutagenic products because there was no effect of the addition of paraoxon, a known esterase inhibitor, on tris-BP mutagenicity.

Fig. 1 shows the effects of cations on the mutagenic potencies of mono-BP and its salts in the presence of S9 mix. The order was mono-BP-NH_4 > mono-BP-Mg > mono-BP. The same order was observed for bis-BP and its salts, as shown in Fig. 2. However, mono-BP and its salts were usually more active than the corresponding bis-BPs independently of the kind of cation. Hedenstedt et al. [5] have reported the effects of metal ions on mutagenicities of dithiocarbamates and have suggested that the metal ion can modify the mutagenic effect, most likely owing to different stabilities of the chelates formed. Phosphoric acid esters are chelating agents, too. Therefore, we think that the effects of cations observed in our experiments also result from the stability of chelates.

In our previous paper [10], DB-1 and DB-2 were reported to show strong

TABLE 2

MUTAGENIC ACTIVITIES OF MONO-BP, BIS-BP AND TRIS-BP WITH STRAIN TA100 OF *Salmonella typhimurium*

Test compound	Dose (μmoles/plate)	Revertant colonies per plate	
		− S9 mix	+ S9 mix
Mono-BP	0	175	169
	1		453
	2		571
	5	241	822
	10	289	1228
	15	300	1427
	20	327	1555
	30	363	1415
	40	393	408
	50	392	274
	60	332	265
Bis-BP	0	174	159
	2	215	299
	4	282	380
	6	293	457
	8	377	515
	10	461	490
	12	573	489
	15	640	458
	20	479	433
Tris-BP	0	184	164
	0.1		433
	0.2		1158
	0 3		2442
	0.4		3660
	0.5		3938
	0 6		4026
	0 8		2910
	1	215	1938
	2	309	1070
	4	503	1130
	6	656	
	8	830	
	10	1008	
	15	1197	
	20	1370	
	30	812	

mutagenicities in the presence of S9 mix. Now, we have clarified that they contained mono- and bis-BP salts but no tris-BP, not only by gas chromatography but also by considering the industrial preparation processes shown in Table 1. Therefore, we compared the mutagenicities of DB-1 and DB-2 with pure mono- and bis-BP salts,

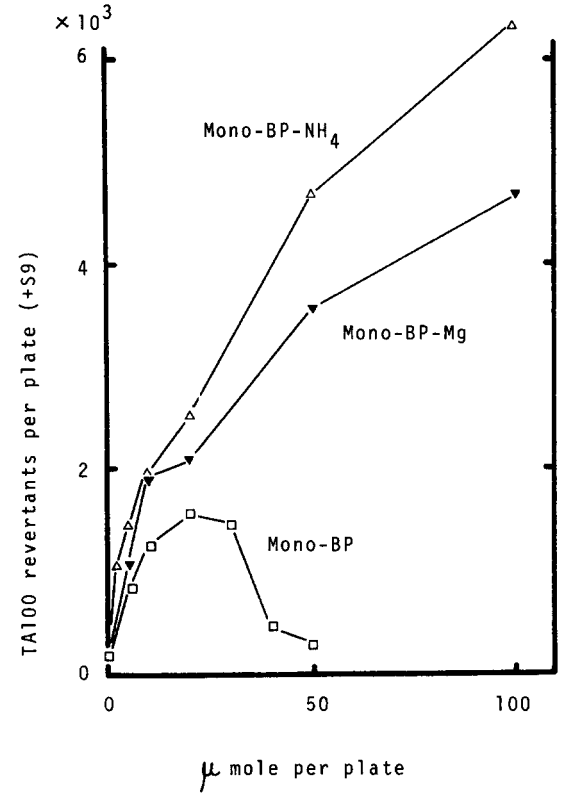

Fig. 1. Mutagenicity of mono-BP and its salts Each point represents the average of duplicate plates

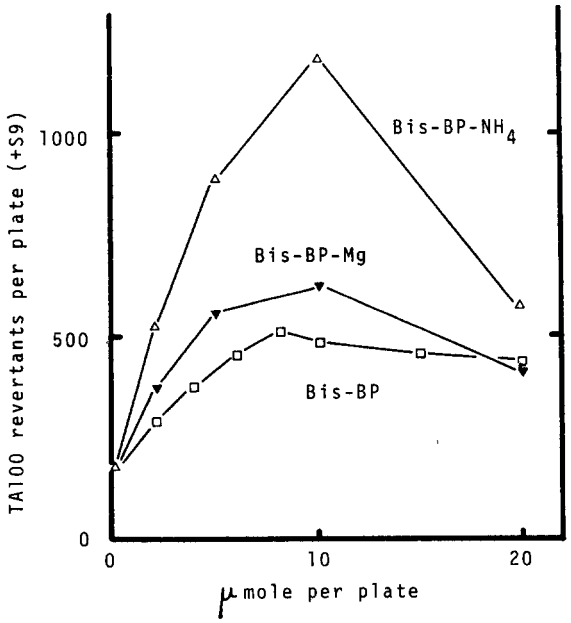

Fig 2 Mutagenicity of bis-BP and its salts Each point represents the average of duplicate plates.

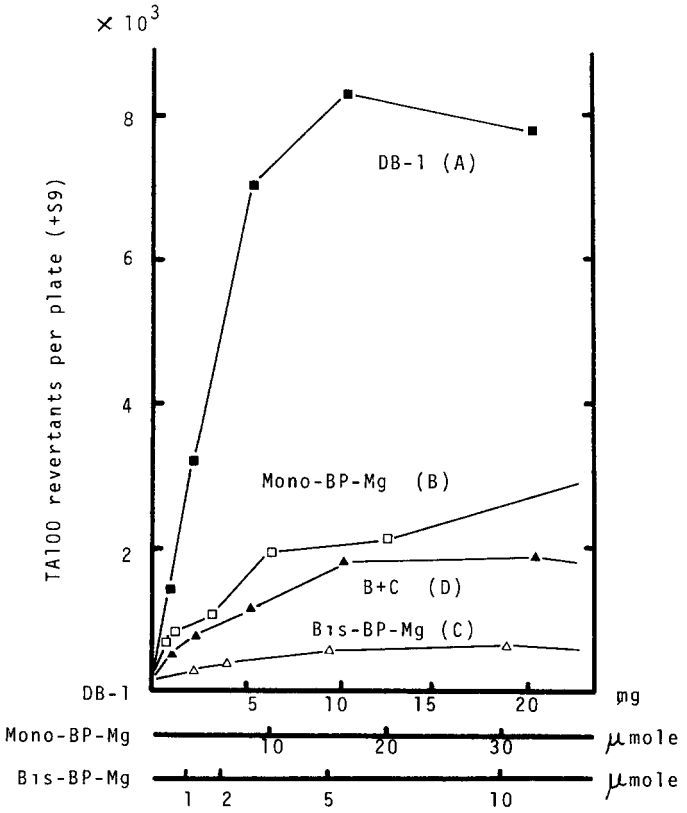

Fig 3. Mutagenicity of DB-1 and its constituents. Each point represents the average of duplicate plates Three horizontal scales (upper, middle, lower) are given. The upper is for DB-1, the middle for mono-BP-Mg and the lower for bis-BP-Mg For example, 5 mg of DB-1 contains 8.06 μmoles of mono-BP-Mg and 2 69 μmoles of bis-BP-Mg

and also with the sum of the corresponding amounts of mono- and bis-BP salts.

Fig. 3 shows the results for DB-1 (A), pure mono-BP-Mg (B) and pure bis-BP-Mg (C) corresponding to the amounts in DB-1, and the sum of mono-BP-Mg and bis-BP-Mg (D). DB-1 was most active, followed by mono-BP-Mg and bis-BP-Mg. No synergistic effect between mono-BP-Mg and bis-BP-Mg was observed, as opposed to our expectations. Moreover, the mutagenicity of the sum of mono- and bis-BP-Mg salts was equivalent to, or slightly weaker than, that of pure mono-BP-Mg. The same tendency was found in the ammonium salt series, as shown in Fig. 4.

Thus, it must be explained that the strong mutagenicities of DB-1 and DB-2 are partially contributed by mono- and bis-BP salts, but mainly caused by other more potent unknown compounds formed during production or storage. We found 3 unknown peaks other than mono- and bis-BPs in the gas chromatogram of DB-2, but no other peaks in that of DB-1. Further studies are necessary to explain the mutagenicities of DB-1 and DB-2.

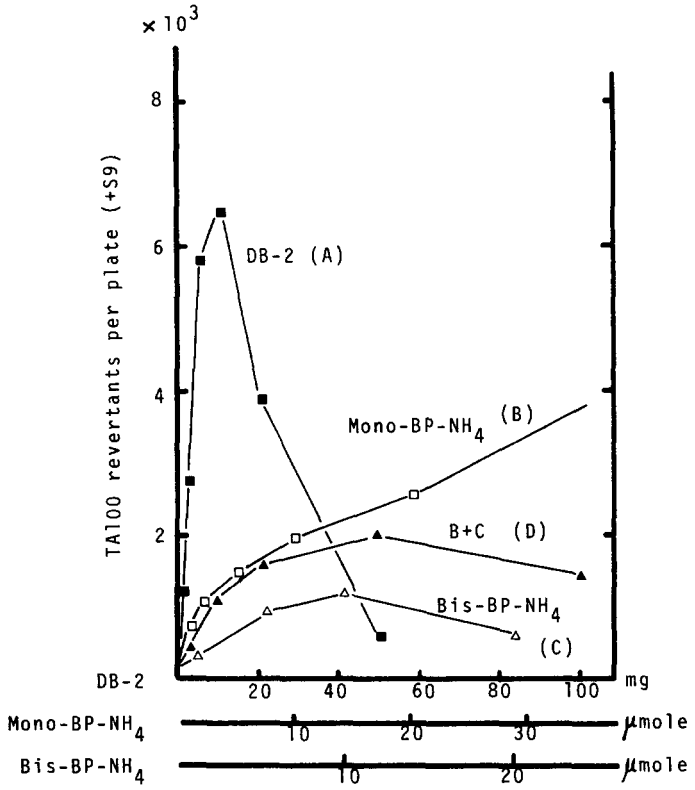

Fig. 4. Mutagenicity of DB-2 and its constituents. Each point represents the average of duplicate plates Three horizontal scales (upper, middle, lower) are given The upper is for DB-2, the middle for mono-BP-NH_4 and the lower for bis-BP-NH_4 For example, 20 mg of DB-2 contains 6.93 μmoles of mono-BP-NH_4 and 4 82 μmoles of bis-BP-NH_4.

References

1 Blum, A., and B.N Ames, Flame-retardant additives as possible cancer hazards, Science, 195 (1977) 17

2 Blum, A., M.D. Gold, B.N. Ames, C. Kenyon, F.R. Jones, E.A. Hett, R.C Dougherty, E.C. Horning, I. Dzidic, D.I. Carroll, R.N. Stillwell and J.P. Thenot, Children absorb tris-BP flame retardant from sleepwear: Urine contains the mutagenic metabolite, 2,3-dibromopropanol, Science, 201 (1978) 1020–1023

3 DiPietro, J., J.H. Todd and R.J. Mulph, Production of flame retardant cellulosic materials, U S Patent, 1972/3 650 820.

4 Federal Register, 42, No 68, 8 April 1977

5 Hedenstedt, A., U. Rannug, C. Ramel and C.A. Wachtmeister, Mutagenicity and metabolism studies on 12 thiuram and dithiocarbamate compounds used as accelerators in the Swedish rubber industry, Mutation Res , 68 (1979) 313

6 IARC, Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 20, (1979) 575–588.

7 Japanese Cabinet Order, No. 334-8-2, revised, 27 August 1981.

8 Lynn, R K , K Wong, R G Dickinson, N Gerber and J M Kennish, Diester metabolites of the flame retardant chemicals, tris(1,3-dichloro-2-propyl)phosphate and tris(2,3-dibromopropyl)phosphate in the rat Identification and quantification, Res. Commun Chem Pathol , 28 (1980) 351–360
9 Nakamura, A , S Kojima and M.-A. Kaniwa, Eiseikagaku, in press.
10 Nakamura, A., N. Tateno, S Kojima, M -A Kaniwa and T Kawamura, The mutagenicity of halogenated alkanols and their phosphoric acid esters for *Salmonella typhimurium*, Mutation Res , 66 (1979) 373–380
11 National Cancer Institute, Bioassay of tris(2,3-dibromopropyl)phosphate for possible carcinogenicity (Technical report series No. 76), Publ. No. (NIH) 78-1326 Washington DC, US Government Printing Office, 1978
12 Ogawa, Y , H Hisada and T. Kasahara, Flame-retardant polyolefin compositions, US Patent 1973/3780142
13 Prival, M.J , E.C. McCoy, B Gutter and H S Rosenkranz, Tris(2,3-dibromopropyl)phosphate Mutagenicity of a widely used flame retardant, Science, 195 (1977) 76
14 Søderlund, E.J., S.D. Nelson and E Dybing, Mutagenic activation of tris(2,3-dibromopropyl)phosphate· The role of microsomal oxidative metabolism, Acta Pharmacol. Toxicol , 45 (1979) 112–121
15 St John, L E , M.E Eldefrawi and D.J. Lisk, Studies of possible absorption of a flame retardant from treated fabrics worn by rats and humans, Bull. Environ Cont Toxicol., 15 (1976) 192–197
16 Suzuki, Y., et al , unpublished data.
17 Van Duuren, B L., G Loewengart, I. Seidman, A C Smith and S. Melchonne, Mouse skin carcinogenicity tests of the flame retardants tris(2,3-dibromopropyl)phosphate, tetrakis(hydroxymethyl)phosphonium chloride, and polyvinyl bromide, Cancer Res., 38 (1978) 3236–3240