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In Vivo Binding of the Flame Retardants *Tris*(2,3-Dibromopropyl) Phosphate and *Tris*(1,3-Dichloro-2-propyl) Phosphate to Macromolecules of Mouse Liver, Kidney and Muscle

Nydia M. Morales[1] and H. B. Matthews[2]

[1]*Department Bioquimica, Esc. Medicina, Universidad de Costa Rica, San Jose, Costa Rica, A.C.*

[2]*NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709*

Federal regulations on flame resistance for children's sleepwear led to the topical application of one or more chemicals to the textiles used in sleepwear in order to confer flame resistance (GUTENMANN & LISK 1975). The most widely used of these flame retardants prior to 1977 was tris(2,3-dibromopropyl) phosphate (Tris). However, the use of Tris as a flame retardant in sleepwear was discontinued in 1977 because it had been shown to be mutagenic in several short-term bioassay systems (PRIVAL et al. 1977, BLUM & AMES 1977, GOLD et al 1978) and carcinogenic in mice and rats (VAN DUUREN et al. 1978, NCI 1978, REZNIK et al. 1979). Dermal application of Tris produced increased tumor incidence in the skin, oral cavity, stomach and lung of female mice (VAN DUUREN et al. 1978) and testicular atrophy and chronic interstitial nephritis in male rabbits (OSTERBURG et al. 1977). Oral administration of Tris resulted in increased tumorigenesis of the liver, lung and stomach of female mice, and increased tumorigenesis of the kidney, lung and stomach of male mice (NCI 1978). In rats, oral administration of Tris increased tumorigenesis of the kidneys of both sexes (NCI 1978, REZNIK et al. 1979).

When the use of Tris in children's sleepwear was discontinued, one of the chemicals used to replace Tris was tris(1,3-dichloro-2-propyl) phosphate (Fyrol FR-2). Due to the structural similarity of Tris and Fyrol and the known mutagenicity of Tris, Fyrol has been the subject of a number of studies to determine its mutagenicity in mammalian and submammalian systems (PRIVAL et al. 1977, GOLD et al. 1978, NAKAMURA et al. 1979). These studies have yielded variable results depending on the type of inducer, microsomal preparation and bacterial strain used, but they have all shown that Fyrol is a much weaker mutagen than Tris. NAKAMURA et al. (1979) did the most thorough study of the mutagenicity of both Tris and Fyrol and their precursors and demonstrated that both the bromine and n-propanol of Tris contribute to its mutagenicity over the chlorine and 2-propanol of Fyrol.

Fyrol has now been voluntarily withdrawn from use as a flame retardant in children's sleepwear, but it was still of interest to know if the structural characteristics which apparently account for the greater mutagenesis of Tris in microbial systems also result in greater covalent binding of Tris to subcellular macromolecules in mammals. Therefore, the present study was designed to compare the covalent binding of Tris and Fyrol to DNA, RNA and protein from

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liver, muscle and kidney of male mice which received a single intravenous dose of one of these flame retardants.

MATERIALS AND METHODS

Tris [U-^{14}C] (specific activity 3.97 mCi/mmole) and Fyrol [U-^{14}C] (specific activity 12.5 mCi/mmole) were purchased from New England Nuclear (Boston, MA). Unlabeled Fyrol was a gift from Stauffer Chem. Corp. (Farmington, CT). Chemical and radiochemical purity of both Fyrol and Tris, as measured by high performance liquid chromatography and thin-layer chromatography was > 99%.

Treatment of Animals. Six weeks old male CD-1 mice (National Institute of Environmental Health Science breeding colony) weighing 30-35 g received a single intravenous dose of either Tris or Fyrol (94.4 μmoles/kg: 376 μCi/kg). Flame retardants were dissolved in Emulphor: ethanol: water (1:1:4) to a final concentration of 19.2 mg/mL. Emulphor EL 620, a polyoxyethylated vegetable oil developed as a vehicle for injection of lipophilic drugs, was a gift from GAF Corp. (New York). Animals were sacrificed 6 h later with ether anesthesia and dissected immediately.

Liver Macromolecules. Each liver was processed separately, and the nucleic acids were extracted with organic solvents and precipitated with ethanol (MORALES & MATTHEWS 1979). The precipitate was resuspended in 0.15 M NaCl, 0.015 M trisodium citrate pH 7.0, and 1/3 of the nucleic acid solution was used for the enzymatic purification of RNA. The RNA thus prepared was primarily rRNA and will be referred to as such. The remainder of the nucleic acid solution was used to separate and purify DNA and low molecular weight RNA (lmw RNA) (MORALES & MATTHEWS 1979). The proteins from the interphase of the phenol extract were precipitated with methanol and purified with organic solvents as described previously, except that in the case of Tris the extraction with acetone had to be repeated 7 times to remove all the free radioactivity.

Kidney and Muscle Macromolecules. Nucleic acids were isolated from kidney and muscle exactly as described for liver except that for the isolation of kidney DNA and lmw RNA, it was necessary to pool the kidneys from three animals. Kidney and muscle protein were extracted exactly as described for liver protein.

The concentration of Tris or Fyrol derived radioactivity in intact tissue was determined by oxidation in a Harvey Biological Oxidizer (R.J. Harvey Instrument Corp., Hillsdale, NY) followed by liquid scintillation counting (MATTHEWS & ANDERSON 1975). The concentration of each macromolecule was determined spectrophotometrically and the radioactivity determined by liquid scintillation counting (MORALES & MATTHEWS 1979). Each vial and its scintillation solution used in this study was precounted for 50 min to establish the background precisely.

RESULTS AND DISCUSSION

Both Tris and Fyrol are metabolized by dealkylation of the phosphate, and most of the halogenated alkyl group thus formed is subsequently metabolized to $^{14}CO_2$ (ULSAMER et al. 1978, NOMEIR & MATTHEWS 1980). Since the ^{14}C-label used in this study was located in the halogenated alkyl groups, it was possible for a portion of the $^{14}CO_2$ formed by metabolism of the respective flame retardants to enter the one carbon pool and thus be incorporated into endogenous organic molecules. Therefore, the binding of Tris and Fyrol was measured at 6 h postadministration to minimize the background due to ^{14}C-incorporation into newly synthesized DNA, RNA or protein. In order to confirm that the radioactivity observed in the subcellular macromolecules was covalently bound, the fractions which contained radioactivity that was resistant to extraction with organic solvents were subjected to chemical hydrolysis and re-extracted as described previously (MORALES & MATTHEWS 1979). The results of these procedures showed that in each instance 95% or better of the radioactivity associated with the respective macromolecule was covalently bound.

Results are presented in Table 1. It should be noted that 6 h after the administration of a single iv dose (94.4 μmoles/kg) of either Fyrol or Tris there were varying amounts of radioactivity in each of the three tissues assayed and in the nucleic acids and protein isolated from each tissue. The highest concentration of Tris derived radioactivity was observed in kidney, whereas Fyrol derived radioactivity was more concentrated in the liver. In each tissue in which it was measured, the highest concentration of bound radioactivity was in lmw RNA and in most instances decreasing concentrations were observed in protein, rRNA and DNA, respectively.

Binding of Fyrol to liver rRNA, protein and lmw RNA was greater than the respective binding of Tris; the greatest difference in the extent of binding was observed in protein and lmw RNA (3.1 and 1.8 fold, respectively). On the other hand, Tris binding to DNA exceeded that of Fyrol by 1.6 fold despite the 1.6 fold higher concentration of Fyrol derived radioactivity in liver, Table 1. In kidney tissue and in all of the macromolecules isolated from kidney tissue the concentration of Tris derived radioactivity exceeded that of Fyrol derived radioactivity. Furthermore, the most striking difference in the amounts of Tris and Fyrol binding was observed in the DNA isolated from kidney, Table 1. DNA from the kidneys of mice which were treated with Tris contained radioactivity equivalent to 11.5 ± 5.0 pmoles of Tris /mg, whereas only unquantifiable traces of Fyrol derived radioactivity were bound to kidney DNA.

Chemical carcinogenesis is thought to be mediated by covalent binding of the carcinogens or their metabolites to one or more subcellular macromolecules (WEISBURGER 1978). Likewise the mutations induced in microbial test systems by chemical carcinogens are mediated by covalent binding of the chemical or one of its metabolites to microbial DNA (DEVORET 1979). NAKAMURA et al. (1979) have demonstrated that Tris and 2,3-dibromopropanol are an order of

TABLE 1. In Vivo Binding of Tris and Fyrol Derived Radioactivity to Subcellular Macromolecules from Various Tissues of Treated Mice[a]

Tissue	Flame Retardant	Intact Tissue (pmoles/	rRNA[b] (pmoles/ mg)	DNA (pmoles mg)	Protein (pmoles/ mg)	lmw RNA[c] (pmoles/ mg)
Liver	Tris	32 ± 3[d]	20 ± 2	13 ± 2	18 ± 9	36 ± 6
	Fyrol	51 ± 4	28 ± 6	8.3 ± 2.3	57 ± 11	67 ± 6
Kidney	Tris	41 ± 4	36 ± 4	11 ± 5	58 ± 2	113 ± 33
	Fyrol	31 ± 8	13 ± 3	<1	43 ± 2	93 ± 31
Muscle	Tris	6.3 ± 2.3	7.4 ± 1.7		1.8 ± 0.3	
	Fyrol	5.2 ± 0.8	5.6 ± 1.3		7.2 ± 0.9	

[a]Adult male mice treated with Tris = [^{14}C] tris(2,3-dibromopropyl) phosphate or Fyrol = [^{14}C] tris(1,3-dichloro-2-propyl) phosphate by iv injection (94.4 µmoles and 376 µCi/kg body wt.) were sacrificed 6 h post-treatment.

[b]Whole cell RNA (see Materials and Methods).

[c]RNA of low molecular weight (see Materials and Methods).

[d]Values represent average ± SD (n=4).

magnitude more mutagenic when activated by the S9 fraction from rat liver than in the absence of the S9 fraction. NAKAMARA et al. (1979) have also reported that Tris and 2,3-dibromopropanol are at least ten times more potent as bacterial mutagens than are Fyrol and 1,3-dichloro-2-propanol which are only weakly mutagenic in the same system. These results indicate that the chemicals which interact with bacterial DNA are metabolites of the respective chemicals tested and that the metabolite(s) of Tris are more potent mutagens than the metabolite(s) of Fyrol. In comparison with the mutagenicity tests, the results of the present study show, with the single exception of binding to kidney DNA, much less dramatic variations in the amount of covalent binding of Tris and Fyrol to subcellular macromolecules (NAKAMURA et al. 1979). However, it should be pointed out that from these preliminary data there is no way of knowing if the assay point chosen for covalent binding, 6 h, is the optimum time to demonstrate relevant differences in the binding of Tris and Fyrol. It is also possible that the covalent binding of Fyrol is at a less critical site or is more easily repaired than is that of Tris. And finally, it is possible that the results of the mutagenicity assays are better indicators of the lesion induced at a specific site of DNA than of the less specific and less hazardous covalent binding to other sites on DNA or other subcellular macromolecules.

The preliminary results presented in this report have demonstrated a potential for each of the compounds studied to bind covalently to subcellular macromolecules of liver and kidney. These are two tissues which have been shown to be sites of increased tumorigenesis induced by the chronic administration of Tris (NCI 1978). But perhaps more important, these preliminary results point out that additional comparative studies of the covalent binding of Tris and Fyrol may provide a greater insight into the structure-activity relationships which determine covalent binding of halogenated phosphate esters. These additional studies should be designed to determine binding at sequential time points, to compare the degree of binding following chronic administration and to determine the rates of repair of covalent binding of each of the flame retardants to both DNA and RNA in order to determine if differences in the covalent binding of these two compounds to subcellular macromolecules are accurately predicted by the results of short-term mutagenicity assays.

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REFERENCES

BLUM, A. & B. N. AMES: Science 195, 17 (1977).
DEVORET, R.: Scientific American 241, 40 (1979).
GOLD, M. D., A. BLUM, & B. N. AMES: Science 200, 785 (1978).
GUTENMANN, W. H. & D. J. LISK: Bull. Environ. Contamin. Toxicol. 14, 61 (1975).
MATTHEWS, H. B. & M. W. ANDERSON: Drug Metab. Disp. 3, 211 (1975).
MORALES, N. M. & H. B. MATTHEWS: Chem. Biol. Interactions 27, 99 (1979).
NAKAMURA, A., N. TATENO, S. KOJIMA, M. KANIWA, & T. KAWAMURA: Mutation Res. 66, 373 (1979).
NATIONAL CANCER INSTITUTE: Bioassay of tris(2,3-dibromoproyl) phosphate for possible carcinogenicity, Technical Report Series No. 76 (1978).
NOMEIR, A. A. & H. B. MATTHEWS: Toxicol. Appl. Pharmacol. In press (1980).
OSTERBERG, R. E., G. W. BIERBOWER, & R. M. HEHIR: J. Toxicol. Environ. Hlth. 3, 979 (1977).
PRIVAL, M. J., E. C. MCCOY, B. GUTTER, & H.S. ROSENKRANS: Science 195, 76 (1977).
REZNIK, G. J. M. WARD, J. F. HARDISTY, & A. RUSSFIELD: J. Natl. Cancer Inst. 63, 205 (1979).
ULSAMER, A. G., W. K. PORTER, & R. E. OSTERBERG: J. Environ. Pathol. Toxicol. 1, 543 (1978).
VAN DUUREN, B. L., G. LOAWENGART, I SEIDMAN, A. C. SMITH, & S. MELCHIONNE: Cancer Res. 38, 3236 (1978).
WEISBURGER, E. K.: Ann. Rev. Pharmacol. Toxicol. 18, 395 (1978).