Studies of Possible Absorption of a Flame Retardant from Treated Fabrics Worn by Rats and Humans

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The use of flame retardants in plastics and textiles in 1975 has been estimated at 3.5-4.5 billion pounds (CHEM. and ENG. NEWS, 1971). Many chemical classes of compounds have been used as flame retardants including organic compounds containing phosphorus, sulfur, halogens and nitrogen and inorganic compounds of antimony, boron and others (LYONS, 1970). Among other methods, topical application of the compound as the monomer is used to flame retard textiles. The coated fabric is then typically heat-treated to facilitate incorporation of the coating into the fabric. To attain satisfactory flame resistance some retardants must be applied at rates up to 35% of the weight of the fabric (CHEM. and ENG. NEWS, 1971).

Organophosphorus compounds are presently being used extensively as flame retardants on fabrics and legally must be used on childrens sleepwear. A promising compound in this group is tris (2,3-dibromopropyl) phosphate (TBPP). Earlier work indicated that appreciable quantities (up to 10 micrograms per square inch of fabric) of this and other organophosphorus flame retardants are released from fabrics during a simulated laundering step and that this rate of release is maintained during several subsequent launderings (GUTENMANN and LISK, 1975). TBPP was also found to be quite toxic to goldfish (GUTENMANN and LISK, 1975). It has an anticholinesterase activity of about 16% of that of the insecticide Tetram (0,0-diethyl-S-(beta-diethylamino)ethyl phosphorothiolate (GUTENMANN and LISK, 1975). Lipophilic organophosphorus ester insecticides are similar in chemical structure to TBPP and are easily absorbed through the skin (O'BRIEN, 1960) and much research is currently underway to define the extent of hazard to farm workers exposed to these insecticides (WARE, et al. 1973; GUNTHER et al., 1973; SERAT, 1973). In the work reported, a study was made of possible diffusion of TBPP from worn fabrics to skin, and its absorption and toxicological significance.

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Bulletin of Environmental Contamination & Toxicology, Vol. 15, No. 2 © 1976 by Springer-Verlag New York Inc.

Experimental

Exposure Studies With Rats And Humans

I. Rat skin exposure to high concentration of TBPP.
An area of two square inches on the back of a male rat (150g) (Lewis strain; Microbiological Laboratories, Baltimore, Maryland) was shaved to expose the skin. One hundred mg of the highly purified liquid TBPP monomer was spread over the surface of the gauze pad of a 1-inch bandage (Band Aid®) and pressed tightly against the shaved skin. The bandage was secured in place by adhesive tape. The animal was then held in an all glass metabolism cage for 7 days during which time total urine was collected daily and frozen prior to analysis.

II. Rat skin exposure to fabric treated with TBPP.
The entire torso of a second rat was shaved. A snug-fitting sleeve was then fashioned from a 4 x 6 inch portion of 100% polyester flannel commercially prepared with a flame retardant finish of TBPP. The sleeve was placed around the animal in direct contact with the skin, 24 hours per day. The animal was then similarly held in the metabolism cage for 9 days and total urine was collected daily for analysis.

III. Human exposure to fabric treated with TBPP.
Pajamas, 100% polyester knit and with a TBPP flame retardant finish were purchased commercially. They were then worn by one of the authors (D.J.L.) and a 5-year old boy for a period of 7 nights. Morning urine samples were collected daily throughout this period and up to 8 days thereafter. In each of the above trials, urine collected prior to exposure to TBPP or TBPP-finished fabrics served as controls.

Analytical Methods

It is well known that following absorption of organophosphorus insecticides by mammalian species they typically undergo enzymatic or chemical hydrolysis to the corresponding acids and alcohols (O'BRIEN, 1960). The alcohols are often excreted in the urine as soluble conjugates. Since hydrolysis of TBPP would yield 2,3-dibromopropanol (DBP), an analytical method was devised to determine the presence of this compound freely or as a conjugate in urine. The method for the determination of free DBP was as follows:

Two grams of urine was transferred to a 50 ml volumetric flask. Five ml of N hydrochloric acid and 5 ml of diethyl ether were added and the contents of the flask was made to volume with saturated sodium chloride and shaken vigorously.

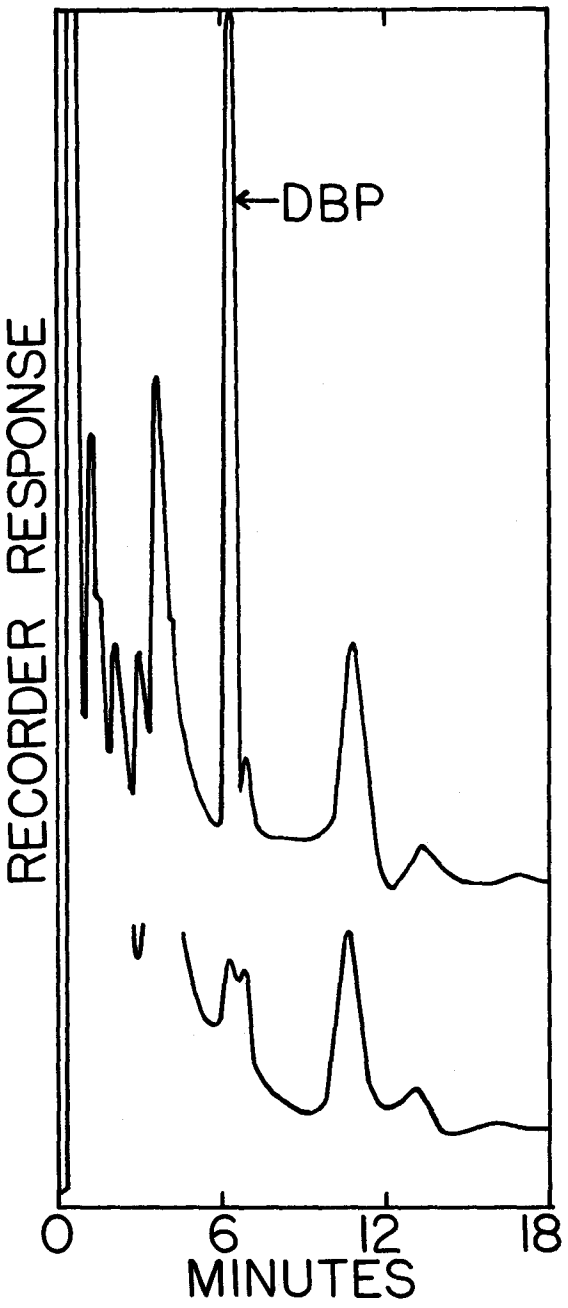

Figure 1. Gas chromatograms (upper) representing the total of free and conjugated DBP eluting as the methyl ether in hydrolyzed rat urine 4 days after dermal application of TBPP and (lower) control urine.

The layers were separated by centrifugation and 2 ml of the ether layer was transferred to a 10 ml volumetric flask. Methanol (0.22 ml) was added and the solution was methylated with diazomethane by the procedure of Schlenk and Gellerman SCHLENK and GELLERMAN, 1960). The resulting methyl ether of DBP was then determined by electron affinity gas chromatography.

The determination of total free and conjugated DBP was conducted as follows:

Two grams of urine was transferred to a concentrating tube. Concentrated hydrochloric acid (0.5 ml) was added, the tube was fitted with a condenser through which ice water was circulated and the contents were refluxed using a boiling water bath for 1 hour to hydrolyze conjugates of DBP. The mixture was transferred to a 50 ml volumetric flask and the remainder of the procedure was the same as described above for analysis of free DBP beginning with addition of diethyl ether. The concentration of conjugated DBP was obtained by difference.

The gas chromatograph was a Barber-Colman Model 10 with a battery operated 6-cc radium-226 electron affinity detector. The recorder was a Wheelco, 0 to 50 mv. with 10-inch chart paper running 10 inches per hour. The column was glass, 7 mm i.d., 6 ft. long, packed with 10% DC-200 on 80 to 100 mesh Gas Chrom Q and operated at 110° C. Nitrogen (60 cc per min) was the carrier gas. The retention time of the methyl ether of DBP was 6 min. The recovery of 5 ppm of DBP added to control urine ranged from 84 to 90%. The limits of detection of the methods for DBP in rat and human urine were, respectively, 0.4 and 0.2 ppm.

Results and Discussion

Table 1 lists the concentrations of free and conjugated DBP which appeared in the urine of the rat to which the pure liquid TBPP was applied as a function of time after dermal application. Figure 1 shows chromatograms of DBP (eluting as the methyl ether) in hydrolyzed rat urine representing both free and conjugated DBP on the fourth day after liquid TBPP was applied to the rats' skin. Appearance of free or conjugated DBP in the urine was slow (Table 1). Urine production also diminished with time after application of TBPP. The weights in grams of total daily urine produced on the respective days after application of TBPP were: day zero (day of TBPP application and control) - 12.2, day one - 8.8, day two - 11.0, day four - 9.2, day five - 6.2 and day seven - 4.1. Higher concentrations of DBP and its conjugates in urine particularly on days 5 and 7 (Table 1) may have been due largely to a concentrating effect of lowered urine output.

Table 1. Concentrations of free and conjugated DBP in rat urine as a function of time after dermal application of liquid TBPP on day zero.

Day	Urinary concentration as TBPP (ppm) DBP (free)	DBP (conjugated)
0 (control)	nd[1]	nd
1	nd	1.28
2	0.80	1.17
4	2.67	2.83
5	12.81	10.67
7	1.33	7.63

[1] not detectable

To study possible hydrolysis of TBPP to DBP in rat liver, TBPP was incubated in vitro for 30 minutes with the 10,000 X g supernatant fraction of fresh rat liver which contains microsomes and soluble enzymes by the standard procedure (GUTENMANN et al., 1972). Gas chromatographic analysis for DBP showed about 5% conversion of TBPP to DBP.

No detectable residues of free or conjugated DBP were found in any of the urine samples of the rat that wore the TBPP-finished polyester flannel sleeve or in the urine of the human subjects that had worn the TBPP-flame retardant pajamas. Small amounts of DBP and conjugates did appear in rat urine when the animal was allowed to chew on the fabric, however. Apparently if there was migration of TBPP from the worn fabrics to the skin followed by absorption and hydrolysis, the quantities were too small to detect. The rate of migration of flame retardants from fabric to skin and the rate of absorption would probably depend on the chemical structure and lipid solubility of the flame retardant, the nature of the fabric, operating conditions during commercial production of the finished fabric, thickness of the flame retardant coating (the evenness of which can vary considerably over various portions of a given fabric) closeness of fit of the fabric to the skin, and differences among individuals (oiliness of or breaks in the skin, etc.). It is also possible that the rate of migration of a flame retardant to skin would be enhanced by conditions which cause profuse sweating as in the case of fever but no data is available to substantiate this. If a child were to chew on parts of a flame-retardant garment, removal and ingestion of the coating would be possible.

Since other commercially used organophosphorus flame retardants such as Pyrovatex CP (N-methylol dimethyl phosphonopropionamide) also show comparable anticholinesterase activity to that of TBPP (GUTENMANN and LISK, 1975) further studies are necessary to accurately assess the safety of these chemical finishes on various fabrics. The lack of analytical methods and pure standards for various of these materials and their hydrolysis products is probably the greatest barrier to further investigations. The synthesis and use of isotopically labelled flame retardants for experimentation would greatly facilitate such studies.

Summary

An investigation was made of the possible migration of a flame retardant, tris (2,3-dibromopropyl) phosphate (TBPP), commercially used in childrens sleepwear, to the skin of rats and humans and subsequent absorption. When the pure chemical was applied directly to the shaved skin of a rat, the hydrolysis product, 2,3-dibromopropanol (DBP), appeared in the urine. The 10,000 X g supernatant fraction of rat liver hydrolyzed TBPP to DBP. When TBPP-finished fabrics were worn by a rat, an adult male and a 5-year old boy for up to 9 days, DBP was not detected in the urine.

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Acknowledgement

The authors thank E. Peters for valuable consultations during this investigation.