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# Mouse Skin Carcinogenicity Tests of the Flame Retardants Tris(2,3-dibromopropyl)phosphate, Tetrakis(hydroxymethyl)phosphonium Chloride, and Polyvinyl Bromide[1]

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## ABSTRACT

**The flame retardants tris(2,3-dibromopropyl)phosphate, tetrakis(hydroxymethyl)phosphonium chloride, and polyvinyl bromide were tested for carcinogenic activity by skin application 3 times weekly in random-bred female ICR/Ha Swiss mice for 420 to 496 days. Tris(2,3-dibromopropyl)phosphate at two dose levels (30 mg and 10 mg/application) induced benign and malignant tumors of the skin, forestomach, and oral cavity (tongue and gingiva) in a statistically significant number of mice (30/group). A statistically significant incidence of papillary tumors of the lung was observed at both dosages. One carcinoma of the liver was observed at both doses, and the higher dose also resulted in one mouse with a tubular adenoma of the kidney. Tetrakis(hydroxymethyl)phosphonium chloride (2 mg/application, 60 mice) and polyvinyl bromide (0.1 ml latex suspension/application, 30 mice) were inactive. Polyvinyl bromide was also injected s.c. into another group of female ICR/Ha Swiss mice once weekly for 48 weeks, and the mice were observed for a total of 60 weeks. Liposarcomas were induced in 19 of 30 mice, which was ascribed to physical carcinogenesis. Appropriate solvent and no-treatment control groups were included.**

## INTRODUCTION

Chemicals added to fabrics during the manufacturing process to impart flame retardancy have become widely used in recent years. This increased use of flame-retardant chemicals was the result of stringent federal regulations concerning the flammability of finished fabrics. The regulations apply to many fabrics, including cotton, rayon, wool, and many synthetic fabrics (4, 10, 24).

TRIS[3] was, until recently, used as a flame retardant mostly for synthetic fabrics, particularly polyesters. THPC was used as a flame retardant for cotton, wool, and rayon (10). Fabrics containing TRIS were banned in early 1977 by the Consumer Product Safety Commission (5, 7), and THPC has been largely replaced by tetrakis(hydroxymethyl)-phosphonium sulfate (11). Other formulations with the tetrakis(hydroxymethyl)phosphonium cation with a variety of anions are also in use (10, 28).

These flame retardants have been extensively used in the past, and the use of TRIS in children's sleepwear has been of particular concern because of the possible deleterious effects of chronic low-level skin exposure of a large population (4, 5, 24).

Flame retardants are added to the fabric in large amounts, up to 35% of the final fabric weight (16). The flame-retardant THPC polymerizes in the fabric in the presence of other additives (16).

In light of the above facts, a number of questions can be raised: (*a*) the amount of free chemicals in the fabric; (*b*) the extent to which the free, *i.e.*, unpolymerized, chemical is leached out during wear and in laundering (TRIS is highly insoluble in water, while THPC is highly soluble in water); (*c*) the possible liberation of formaldehyde and hydrochloric acid from free THPC in the fabric (2, 13–15) [formaldehyde and hydrochloric acid are known to be in equilibrium with the animal and human carcinogen bis(chloromethyl)ether (14, 26, 27)]; and (*d*) the skin carcinogenicity, systemic absorption, and hence potential systemic carcinogenicity of these chemicals. These questions have, to date, been answered only in part and will be discussed below.

Studies on TRIS, THPC, and other flame retardants have been under way in this laboratory since 1973, and the work has resulted in several reports from our laboratory (12–15). Recent reports from other laboratories have appeared showing that TRIS is mutagenic in bacterial systems (3, 19). THPC was inactive as a mutagen in 1 of the same systems (B. N. Ames, personal communication). Recently, it was reported that TRIS is carcinogenic when fed to mice and rats (5, 7). That report is, at the time of this writing, not yet in the scientific literature, but it led to the banning of fabrics containing TRIS (5, 7).

PVBR is presently being considered for commercial use as a flame retardant (28) but is, to our knowledge, not yet in use.

In this study only mouse skin was used for the site of application of TRIS and THPC. This is important, since skin is the primary exposure route in humans. Commercial preparations were used purposely, since these are the materials to which humans may have been exposed. A random-bred strain of female ICR/Ha Swiss mice was used in our work rather than the inbred strains of mice and rats used in the earlier work on TRIS (7).

The structures of TRIS, THPC, and PVBR are shown in Chart 1.

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[3] The abbreviations used are: TRIS, tris(2,3-dibromopropyl)phosphate; THPC, tetrakis(hydroxymethyl)phosphonium chloride; PVBR, polyvinyl bromide.

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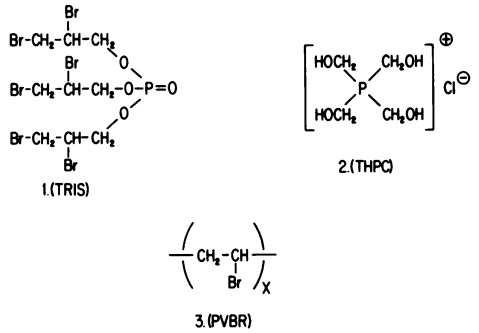


Chart 1. Chemical structures of flame retardants examined.

## MATERIALS AND METHODS

**Chemicals.** Commercial THPC (Hooker Chemical Co., Niagara Falls, N. Y.) is a light-yellow, clear, viscous liquid containing 81% solids. Commercial TRIS (K & K Laboratories, Plainview, N. Y.) is also a light-yellow, clear, viscous liquid of 97% purity. The known impurities in commercial TRIS are 2,3-dibromopropanol, 1,2,3-tribromopropane, and 1,2-dibromo-3-chloropropane (3). Commercial PVBR (Ethyl Corp., Detroit, Mich.) is a 40% aqueous suspension in which PVBR constitutes about 90% of the solids. All 3 materials were used as such for the bioassays in appropriate vehicles, as described under "Results."

**Animals and Bioassay Procedures.** Female ICR/Ha Swiss mice (ARS/Sprague-Dawley Division, The Mogul Corp., Madison, Wis.) were acclimated to laboratory conditions for 2 weeks, and treatments were begun when they were 6 to 8 weeks old. The mice were housed 6/cage on sterile, hardwood chips (Betta Chip; Fisher & Son, Bound Brook, N. J.) in stainless steel cages, fed Purina laboratory chow and water *ad libitum*, and weighed monthly. The animal rooms were maintained at 22 to 24°. Group sizes are listed under "Results." The dosages used in all experiments were based on short-term toxicity evaluations (4 to 6 weeks); the highest possible doses that gave minimal cytotoxic effects, as determined by histopathological examination, were used for the chronic exposures. All compounds were used in well-ventilated hoods, and the mice were housed there for at least 2 hr after treatment. All skin treatments were continued for the duration of the experiment. Animals were examined regularly and scored, and the findings were charted once monthly. Animals in poor health or with large tumor masses were killed. Except for the cranial region, animals were completely autopsied at the end of the experiment or at death. Samples of all abnormal-appearing tissues and organs were excised for histopathological diagnosis. In addition, routine histopathology was performed on skin, liver, and kidney in all animals dying during the test period and in 20% of all animals killed at the end of the test. All tissue sections were fixed in 10% formalin, processed, blocked in paraffin, and stained with hematoxylin and eosin for histopathological examination. Included in the protocols were groups given vehicle only; no treatment groups were included in the protocols.

In the skin application experiments, the dorsal skin of the mice was shaved initially and, when necessary, throughout the test. All compounds were applied in 0.1 or 0.2 ml of solvent (see "Results") to the shaved dorsal skin 3 times/week with a micropipet (Biopette; Carworth Farms, Inc., New City, N. Y.). Skin lesions were diagnosed clinically as papillomas when they reached 1 cu mm in size and persisted for 30 days or more or when they were confirmed histopathologically.

In the s.c. injection experiment, used only for PVBR, the mice were given weekly injections in the left flank with the use of a 26 gauge, 3/8-inch stainless steel needle (Becton, Dickinson and Co., Rutherford, N. J.) mounted on a 1-ml glass tip tuberculin syringe graduated to 0.01 ml. PVBR was injected as an aqueous suspension (described above). Injections (0.05 ml, ~23 mg PVBR) were given for 48 weeks, and the mice had been observed for 60 weeks when the test was terminated.

## RESULTS

The results of the mouse skin experiments with all 3 flame retardants are given in Table 1. THPC and PVBR were both inactive on skin; TRIS induced not only skin tumors in a significant ($p < 0.0005$) number of mice but also substantial numbers of tumors at distant sites. Some of these tumors, notably squamous cell carcinoma of the tongue (Figs. 1 and 2) and squamous cell carcinoma in the gingival area, have never been seen in any of our control or treatment animals involving many chemicals.

The incidence of stomach papillomas was significantly increased by TRIS skin application. One important result regarding the stomach cancers concerns the time to first tumor, which was 21 test weeks for the TRIS low dose (10 mg). In our experience 21 weeks is early for a squamous cell carcinoma of this size (14 x 19 x 30 mm) to appear. A clear dose-response effect was observed for TRIS at 2 dose levels (see Table 1).

The $p$ values given in the table were assigned by means of computation of $\chi^2$ analyses (20, 21) in which the tumor incidences in the test groups were compared with a composite of 4 no-treatment control groups that were used for these tests as well as for other concurrent experiments (249 mice).

In the s.c. injection experiment with PVBR, reported in summary form elsewhere (25), 19 of 30 mice bore liposarcomas, which was ascribed (25) to physical carcinogenesis (9), because the commercial preparation is an aqueous suspension containing 90% solids.

## DISCUSSION

Widespread publicity concerning the mutagenicity of TRIS in *Salmonella typhimurium* (3, 19) and, more recently, its carcinogenicity in mice and rats (7) has caused a great deal of public concern and confusion. The use of inbred strains of mice [C57BL/6 × C3H $F_1$ (hereafter called B6C3$F_1$)] and rats (Fischer 344) in the reported studies (7) has been questioned because of the high incidence of spontaneous tumors in these strains (1). At the same time the use of a wide range of other flame retardants (10) has received scanty attention, particularly with regard to their potential carcinogenicity.

Commercial grade TRIS contains the impurities 2,3-dibromopropanol, 1,2,3-tribromopropane, and 1,2-dibromo-

Table 1

*Carcinogenicity of flame retardants applied to mouse skin*

Compounds were applied to the dorsal skin of female ICR/Ha Swiss mice at the doses indicated 3 times weekly for the duration of the test.

| Treatment (dose) | Days on test | Median survival time (days) | Effective no. of mice[a] | No. of mice with tumors | $p^b$ |
|---|---|---|---|---|---|
| TRIS (30 mg/0.2 ml acetone) | 474 | 383 | 30 | 5 dorsal skin (3 papillomas, 1 carcinoma, and 1 sarcoma) | <0.0005 |
| | | | | 28 lung (papillary tumors) | <0.0005 |
| | | | | 1 liver (carcinoma) | <0.025 |
| | | | | 20 forestomach (13 papillomas and 7 carcinomas) | <0.0005 |
| | | | | 4 oral cavity (2 carcinomas of tongue and 2 carcinomas of gingiva) | <0.0005 |
| | | | | 1 kidney (tubular adenoma) | <0.025 |
| TRIS (10 mg/0.2 ml acetone) | 496 | 425 | 29 | 2 dorsal skin (1 papilloma and 1 carcinoma) | <0.0005 |
| | | | | 26 lung (papillary tumors) | <0.0005 |
| | | | | 1 liver (carcinoma) | <0.025 |
| | | | | 10 forestomach (7 papillomas and 3 carcinomas) | <0.0005 |
| | | | | 2 oral cavity (1 papilloma and 1 carcinoma of gingiva) | <0.0005 |
| THPC, (2 mg/0.2 ml acetone:water; 90:10) | 496 | >496 | 59 | 17 lung (papillary tumors) | NS |
| | | | | 1 forestomach (papillomas) | NS |
| PVBR (0.1 ml)[c] | 420 | >420 | 29 | 7 lung (papillary tumors) | NS |
| Acetone (0.1 ml) | 424 | >424 | 29 | 7 lung (papillary tumors) | |
| | | | | 1 liver (undifferentiated malignant tumor) | |
| | | | | 1 forestomach (papilloma) | |
| Acetone:water (0.1 ml, 90:10) | 365 | >365 | 29 | 7 lung (papillary tumors) | |
| No treatment | 400–649 | >400 | 249 | 90 lung (papillary tumors) | |
| | | | | 7 forestomach (6 papillomas and 1 carcinoma) | |

[a] Effective number of mice includes only those necropsied.
[b] Values > 0.05 are indicated as NS (not significant).
[c] Latex suspension; see text.

3-chloropropane. All 3 compounds are known to be mutagenic in bacterial systems (3), and 1,2-dibromo-3-chloropropane has also been shown to cause a high incidence of squamous carcinoma of the stomach in mice and rats by gastric intubation (17). We have recently tested 1,2-dibromo-3-chloropropane by means of skin application in mice. This study is still under way, but the results to date (440 days) indicate a high incidence of stomach tumors in the test groups (Van Duuren, unpublished data).

Dermal application of pure TRIS to rabbits for 3 months resulted in testicular atrophy and renal damage in males but did not result in any adverse effects in females (18). [$^{14}$C]TRIS, when applied to the skin of rats and rabbits, has been shown to concentrate in the kidney, and most of the TRIS excreted was found in the urine (6, 23).

This report shows that commercial TRIS causes malignant tumors at a number of sites after prolonged skin application at 2 doses. The high incidences of papillomas and carcinomas of the forestomach ($p < 0.0005$) observed in our study, *i.e.*, skin application, correlate with that reported in the feeding experiments (7). Tubular cell adenomas of the kidney (not statistically significant) were observed in female B6C3F$_1$ mice by feeding (7) and in the random-bred ICR/Ha Swiss mice used in this study ($p < 0.025$).

Animals are known to groom themselves and each other, and this is borne out by the appearance of squamous carcinomas of the tongue and gingiva. On the basis of the earlier reports concerning absorption through the skin of animals (6, 23) and the tumor incidences reported in this study, it is clear that skin absorption, as well as animal grooming, play a role in the induction of tumors at sites distant from the site of application.

The long-term application of TRIS also caused the induction of skin papillomas and carcinomas ($p < 0.0005$). This raises the question of the mode of action of TRIS, *i.e.*, whether it must be metabolized to an activated carcino-

genic intermediate or whether it is a direct-acting carcinogen. This is currently under study in our laboratory (B. L. Van Duuren, unpublished data).

THPC was of interest to us because of the possible formation of the animal and human carcinogen bis(chloromethyl)ether from it (14, 15, 26, 27). However, this carcinogen could not be detected in commercial THPC or in extracts of THPC-treated cloth (14). The limit of detection in these experiments was 0.1 ppm. THPC is not mutagenic in *S. typhimurium* (B. N. Ames, personal communication). It has been shown in earlier work from this laboratory (12) to react with guanosine at the 2-amino position to give *N*-{[bis(hydroxymethyl)phosphino]methyl} guanosine. The mechanism by which this compound is formed was given in the same report (12). In another recent study the reactions of THPC with other organic bases are described (8).

In a preliminary mouse skin experiment (14), application of THPC resulted in 1 of 20 female ICR/Ha mice with a squamous carcinoma of the skin. Commercial THPC was applied at a dose of 2 mg in 0.1 ml dimethyl sulfoxide, 3 times weekly for 400 days. The experiment described in this report with a larger group of animals at the same dose but in acetone:water (9:1) as solvent did not result in any skin tumors. No internal tumors were observed that suggested any statistical significance when compared with controls. The difference in results between these 2 experiments must be ascribed to the difference in solvents, since dimethyl sulfoxide has been shown to have unusual effects in mouse skin carcinogenesis (22).

THPC has been replaced commercially by tetrakis(hydroxymethyl)phosphonium sulfate (11), the sulfate salt of the same phosphonium cation shown in Structure 2 on Chart 1. Since chloride ion occurs in both perspiration and urine, this change from THPC to the corresponding sulfate bears no relationship to its potential carcinogenicity or tumor-promoting activity (14).

Commercial PVBR is inactive as a mouse skin carcinogen, and the liposarcomas induced by s.c. injection can probably be ascribed to physical carcinogenesis induced by the emulsion (9, 25). The internal tumors observed in the PVBR-treated animals were not significantly different from those observed in controls, which is important, since this was the purpose of the experiment.

In conclusion, it is urged that the wide range of commercial flame retardants currently in use be carefully scrutinized, beginning with an examination of chemical structure, mutagenicity, and, where indicated, long-term skin carcinogenicity bioassays in rodents. More attention must be given to the occupational exposure of workers handling these flame retardants during their manufacture, as well as during application and the curing of fabrics.

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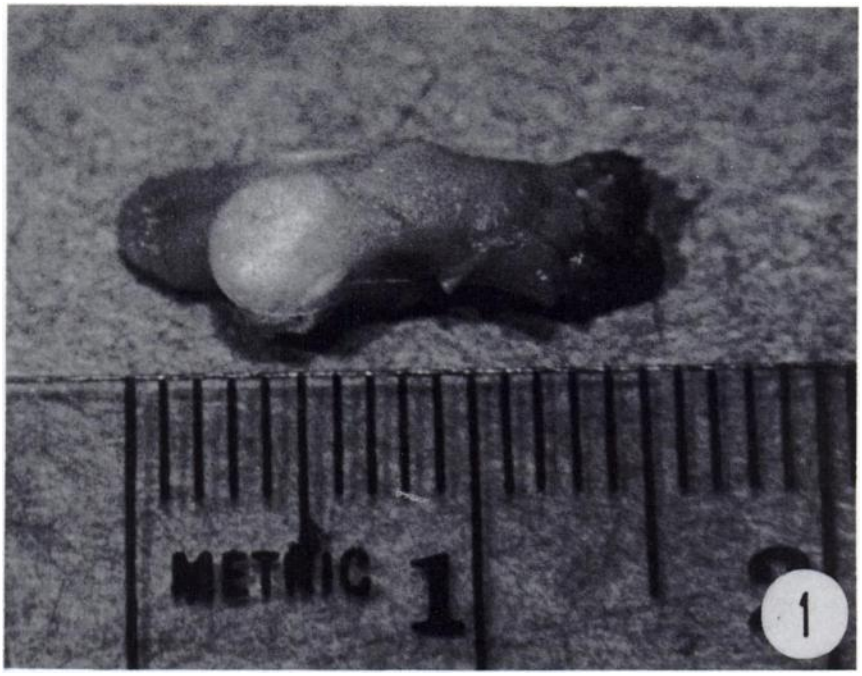


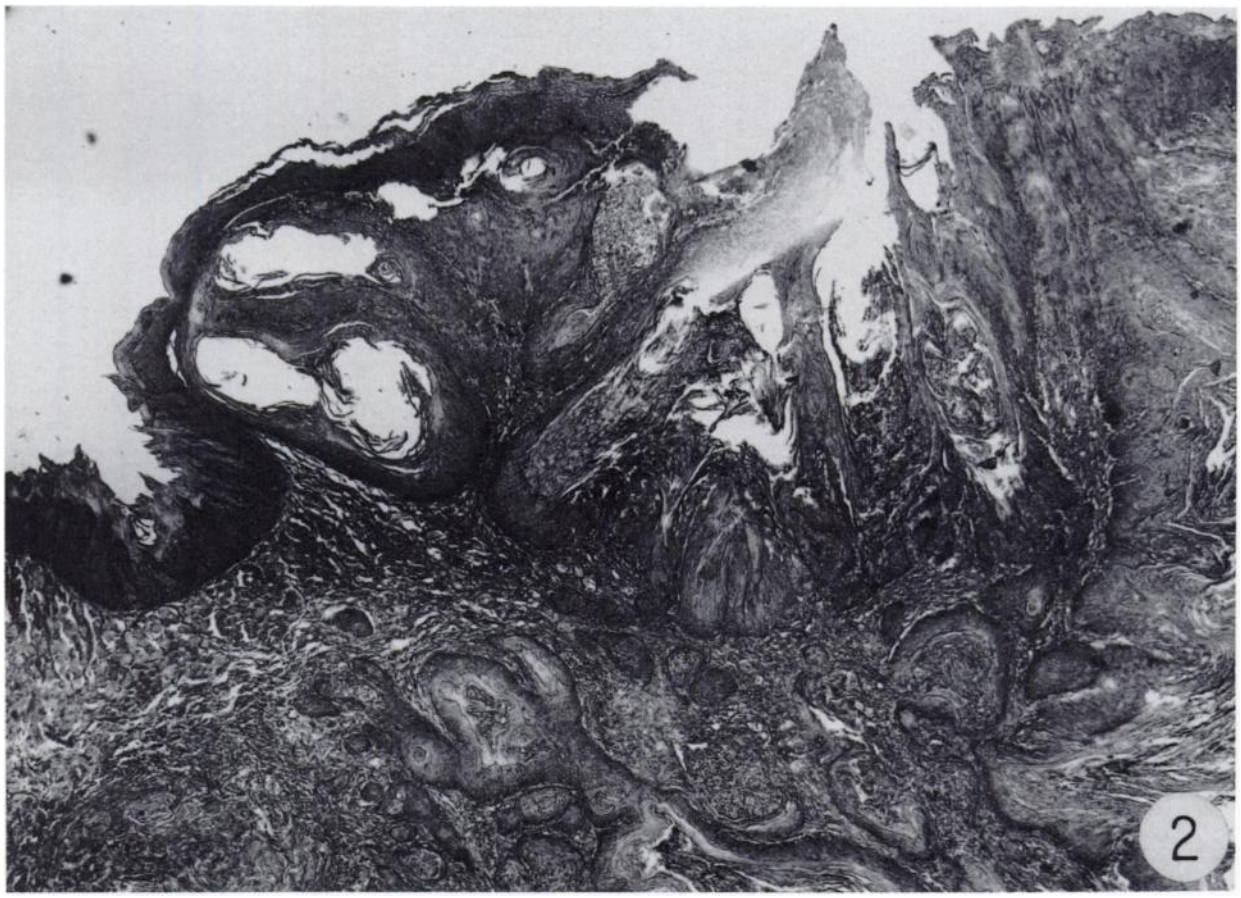


Fig. 1. Squamous cell carcinoma of the tongue in a mouse treated topically with TRIS.
Fig. 2. Squamous cell carcinoma of the tongue shown in Fig. 1. × 40.