

and was not converted to nucleotides efficiently. Also, the data indicate that HPPR monophosphate may act as an analog of inosinic acid which can be converted by adenylosuccinate synthetase to APPR monophosphate in the parasite. The amination of HPPR monophosphate in *T. cruzi* is a reaction which does not occur in mammalian cells (7).

Since HPP must be converted to a ribonucleotide to be active, the reversal of the inhibition by hypoxanthine may be explained as a result of competition of the two bases for the hypoxanthine-guanine phosphoribosyltransferase. Alternatively, the inosinic acid formed from hypoxanthine may compete with HPPR monophosphate for adenylosuccinate synthetase. The reversal of allopurinol's activity by adenine could be due to a preliminary deamination of adenine to hypoxanthine with an action as described, or its activation by an adenine phosphoribosyltransferase to adenosine monophosphate which could compete with APPR monophosphate for conversion to the triphosphate and incorporation into RNA. The obverse, activation of APP by an adenine phosphoribosyltransferase, would not be antagonized by hypoxanthine or inosine and the product, APPR monophosphate, would be directly available for conversion to the di- and triphosphate forms and introduction into cellular RNA. Adenine would compete with APP for the phosphoribosyltransferase or as a nucleotide into cellular RNA. This proposed scheme is summarized in Fig. 3.

Thus, *T. cruzi*, like the pathogenic leishmaniae, appears to possess a phosphoribosyltransferase capable of converting HPP efficiently to HPPR monophosphate and an adenylosuccinate synthetase which can aminate HPPR monophosphate to APPR monophosphate. *Trypanosoma cruzi*, because of enzymatic differences between itself and its host, transforms a compound which is innocuous for mammalian cells into one which is toxic for the parasite. It will be of considerable interest to attempt to exploit these enzymatic differences in the chemotherapy of Chagas' disease.

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Children Absorb Tris-BP Flame Retardant from Sleepwear: Urine Contains the Mutagenic Metabolite, 2,3-Dibromopropanol

Abstract. *The flame retardant, tris(2,3-dibromopropyl)phosphate (tris-BP), which is a mutagen and causes cancer and sterility in animals is absorbed from fabric by people. 2,3-Dibromopropanol, a metabolite of tris-BP and a mutagen itself, has been found in the urine samples of ten children who were wearing or who had worn tris-BP-treated sleepwear. Eight of these children were wearing well-washed sleepwear and the possibility of absorption of tris-BP from well-washed sleepwear is discussed. 2,3-Dibromopropanol was not found in the urines of one child and one adult who had never worn tris-BP-treated garments.*

To evaluate the hazard to human health posed by a chemical, both its toxic effects and human exposure levels should be defined. We now report human absorption of the flame retardant tris-BP, the major chemical used in sleepwear between 1973 and 1977 for the purpose of complying with federal regulations designed to reduce burn injuries in children. About 50 million children were exposed to this chemical before it was banned from use in children's clothing in April 1977. Amounts of about 5 percent of fabric weight had been padded onto children's sleepwear fabric.

Tris-BP produces mutations in bacteria (1, 2), cancer when fed to rats and

mice (3) or when painted on the skin of mice (4), and testicular atrophy and sterility in rabbits after application to the skin (5). Similar chemicals have long been known to penetrate human skin (6), and fabric treated with tris-BP causes an allergic reaction in previously sensitized individuals (7). Radioactivity (¹⁴C) from labeled tris-BP has been found in urine, feces, and body organs of rabbits exposed to fabric treated with tris-BP (8), and the mutagenic tris-BP metabolite, 2,3-dibromopropanol, has been found in urine of animals dermally exposed to tris-BP (9, 10).

Human skin absorption of tris-BP was not found in a previous study in which 2,3-dibromopropanol could be detected at a level of 200 ng/ml (10). However, a rough estimate, based on human absorption studies with structurally similar compounds, suggested that a child wearing sleepwear treated with tris-BP might absorb a considerable daily dose and that doses sufficient to give levels of less than 200 ng/ml in the urine may pose a considerable risk (11). Using a very sensitive analytical method for brominated compounds—negative ion mass spectrometry (12–15)—we have now found 2,3-dibromopropanol (up to 29 ng/ml) in the urine of children wearing tris-BP-treated sleepwear.

Morning urine samples were collected from a 7-year-old female child who had been wearing repeatedly washed sleepwear, some of which had originally been treated with tris-BP. The child then wore new tris-BP-treated pajamas (100 percent polyester) for 5 nights (16). Morning urine samples were collected during the corresponding 5 days and then for the

Table 1. Morning urine samples were obtained from a 7-year-old child. On days 1 and 2 and 8 to 12 the child was wearing repeatedly washed sleepwear that may have been tris-BP-treated; on days 3 to 7 she was wearing new tris-BP-treated pajamas. Urine samples were hydrolyzed with glucuronidase, an enzyme mixture containing glucuronidase and sulfatase, extracted with ethyl acetate, and analyzed by negative ion atmospheric pressure ionization mass spectrometry.

| Day | New treated pajamas | Dibromopropanol (ng/ml) |
|-----|---------------------|-------------------------|
| 1 | No | 0.4 |
| 2 | No | 0.4 |
| 3 | Yes | 11 |
| 4 | Yes | 29 |
| 5 | Yes | * |
| 6 | Yes | 21 |
| 7 | Yes | 18 |
| 8 | No | 9 |
| 9 | No | 14 |
| 10 | No | 6 |
| 11 | No | 6 |
| 12 | No | 8 |

*The urine was lost.

subsequent 5 days when she wore untreated sleepwear.

Analysis of this urine for tris-BP metabolites was carried out by two different procedures (by two separate groups) for sample preparation and negative ion mass spectrometric analysis. One group (Florida) used acid hydrolysis and negative chemical ionization mass spectrometric techniques to provide molecular identification and order-of-magnitude estimation of quantities of tris-BP metabolites in urine (12). The other group (Texas) used enzymatic hydrolysis and negative ion atmospheric pressure ionization mass spectrometric methods allowing for quantitative estimation of amounts of metabolites in urine (13-15).

In the former procedure, 3 ml of urine was acidified to pH 1 with sulfuric acid and heated for 1 hour at 80°C. The sample was then extracted three times with glass-distilled hexane that con-

tained 15 percent 2-propanol. The combined extracts were washed with distilled (three times) water, dried with magnesium sulfate, and reduced in volume with a Snyder column. Spectra were obtained with an AEI MS902 mass spectrometer (12). The methylene chloride-negative chemical ionization mass spectrum of extracts of the child's urine indicated a Br₂Cl isotope cluster at *m/z* 251 to 257. This cluster corresponded exactly to the most intense ions in the mass spectrum of reference 2,3-dibromopropanol, indicating the presence of this tris-BP metabolite in the child's urine.

In the latter quantitative procedure, 1 ml of urine was transferred to a 12-ml screw-capped (Teflon liner) centrifuge tube, and 0.1 g of sodium acetate was added; the pH was adjusted to 4.5 with glacial acetic acid. Glusulase (0.2 ml, Endo) was added, and the hydrolysis was carried out at 37°C for 16 hours with

slow mechanical shaking. The mixture was transferred to another 12-ml screw-capped centrifuge tube, and solid ammonium carbonate was added to saturation. Ethyl acetate (1 ml) and the internal standard (50 ng of 1,4-dibromo-2-butanol in 10 μl of solvent) were added, and the contents of the tube were mixed by inversion and by agitation with a Vortex mixer for 20 seconds. The phases were separated by centrifugation (10 minutes) and the organic phase was removed for analysis. It was not necessary to dry the solution. The solvents used in identification studies were iso-octane and ethyl acetate; ethyl acetate was used in quantitative work because it is more effective as an extracting solvent.

The analytical system consisted of a gas chromatograph (glass column, 1.8 m by 2 mm, inside diameter, with 5 percent SE-30 on 100-120 mesh Gas-Chrom Q as the packing) coupled to an atmospheric

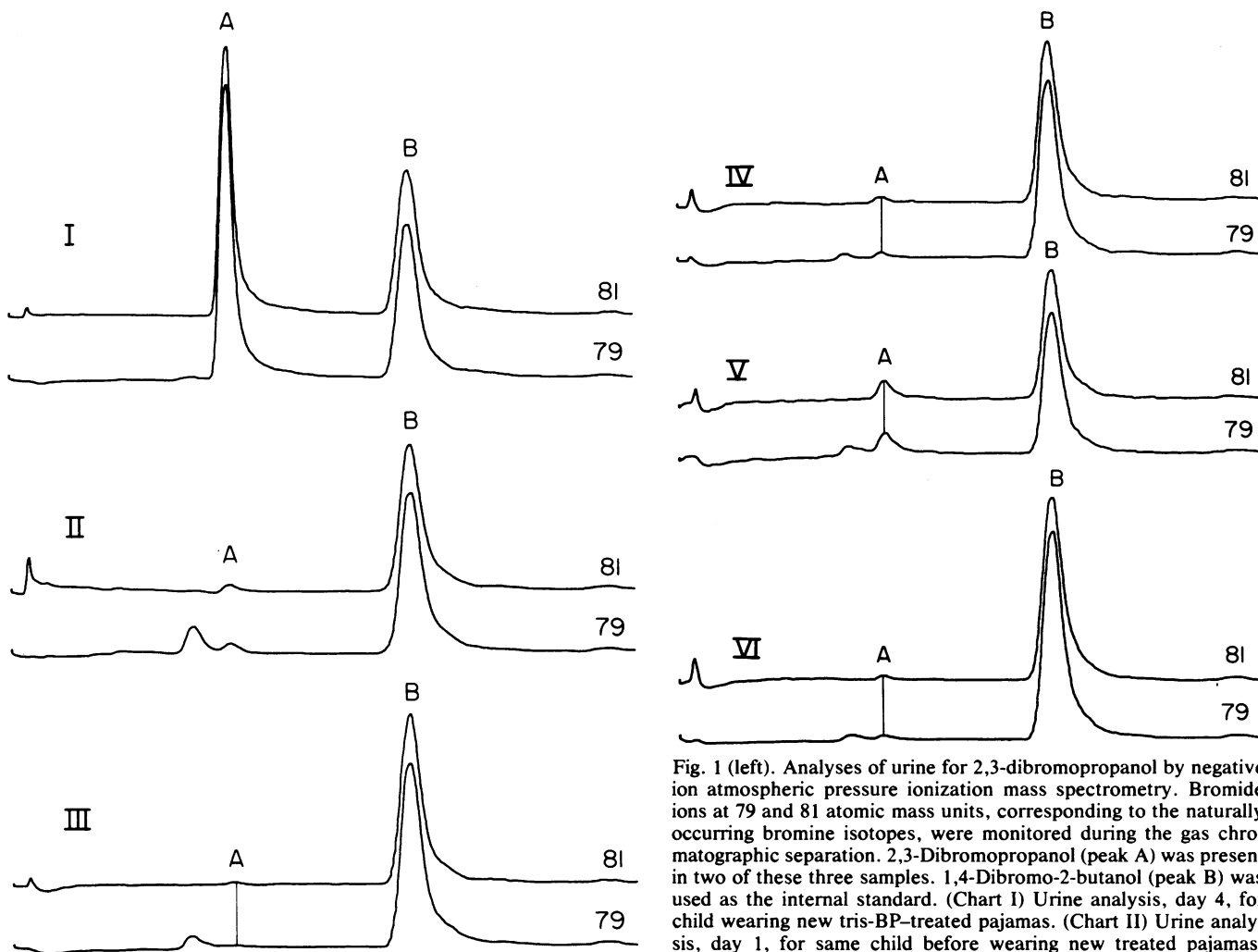


Fig. 1 (left). Analyses of urine for 2,3-dibromopropanol by negative ion atmospheric pressure ionization mass spectrometry. Bromide ions at 79 and 81 atomic mass units, corresponding to the naturally occurring bromine isotopes, were monitored during the gas chromatographic separation. 2,3-Dibromopropanol (peak A) was present in two of these three samples. 1,4-Dibromo-2-butanol (peak B) was used as the internal standard. (Chart I) Urine analysis, day 4, for child wearing new tris-BP-treated pajamas. (Chart II) Urine analysis, day 1, for same child before wearing new treated pajamas. (Chart III) Urine analysis for a child who had never been exposed to

flame-retardant sleepwear. The results indicate that prior exposure to tris-BP had occurred for the child whose analyses are in charts I and II, and that (Table 1) the wearing of new tris-BP-treated pajamas caused a marked increase in the urinary excretion of 2,3-dibromopropanol. Fig. 2 (right). Analyses of urine for 2,3-dibromopropanol by the method used for Fig. 1. Charts IV and V are for different children in different families. Both were wearing treated pajamas that had been laundered for at least 5 months. The urinary excretion of 2,3-dibromopropanol is between 0.5 ng/ml (chart IV) and 5 ng/ml (chart V) under these circumstances. (Chart VI) Urine analysis for child 6 months after cessation of wearing tris-BP-treated pajamas showing a trace level of 2,3-dibromopropanol.

pressure ionization mass spectrometer (13-15). The carrier gas (Linde high purity nitrogen) was passed through an oxygen trap prior to use. The analyses were made isothermally with a column temperature of 110°C (injector temperature 250°C), a detector (source) temperature of 250°C, and a flow rate of 40 ml/min. Two channels of selected ion monitoring were recorded for each analysis. The ions monitored were at 79 and 81 amu, corresponding to bromine isotopes. For each analysis, 4 µl of an ethyl acetate extract of hydrolyzed urine was injected. The limit of detection of dibromopropanol in the urine was 0.1 ng/ml.

Analysis showed that 2,3-dibromopropanol was present at 0.4 ng/ml in the child's urine prior to her wearing the new tris-BP-treated pajamas. Considerably more tris-BP metabolite was found in urine collected both while and after she wore the new pajamas. See Table 1 and Fig. 1 for 2,3-dibromopropanol measured in the child's urine by the Texas group. Values of the same magnitude were obtained by the Florida group.

Urine samples were collected from ten other children and one adult in September and October 1977, about 5 months after the sale of new tris-BP-treated pajamas was banned. All samples were analyzed for 2,3-dibromopropanol, which was not found in urine of the one child and one adult who had never worn flame-retardant sleepwear. Eight children were wearing well-washed tris-BP-treated sleepwear: seven had levels of approximately 0.5 ng of 2,3-dibromopropanol per milliliter in their urine (Fig. 2, chart IV) and one child had 5 ng/ml (Fig. 2, chart V). A trace of 2,3-dibromopropanol was found in the urine of one child who was reported to have stopped wearing tris-BP-treated sleepwear 6 months earlier (Fig. 2, chart VI). Brominated organic chemicals are not naturally found in mammals, and sources of this 2,3-dibromopropanol other than tris-BP are unknown.

Quantitative estimation of the amount of tris-BP absorbed from sleepwear is difficult. A daily amount of 12 µg of 2,3-dibromopropanol excreted in the child's urine can be estimated (17) if we assume a constant level of 20 ng of 2,3-dibromopropanol per milliliter. The amount of tris-BP absorbed is likely to be considerably higher than indicated by the 2,3-dibromopropanol level in urine. This level only reflects tris-BP metabolites excreted in urine and does not take into account the fraction of the chemical or its metabolites that is stored, or excreted, in the feces. The data in our short-term study indicate that 2,3-dibromopropanol

was still being excreted 5 days after the child stopped wearing the new treated sleepwear.

An estimate of the amount of tris-BP absorbed, relative to the amount of 2,3-dibromopropanol found in urine, may be made based on gavage studies carried out at the Consumer Product Safety Commission (CPSC). When rats were dosed with ¹⁴C-labeled tris-BP (50 mg/kg), 6.5 percent of the label was found associated with 2,3-dibromopropanol and its conjugates in the urine (18). Thus the amount of tris-BP absorbed could be as much as 15 times as high as the amount of 2,3-dibromopropanol found in urine. This suggests that the child wearing the new tris-BP-treated sleepwear (Table 1) may have been absorbing a dose of tris-BP of approximately 180 µg/day or 9 µg per kilogram of body weight.

The 7-year-old girl wearing the new pajamas reported that she did not chew her sleepwear. A higher exposure is likely for children who put their sleepwear into their mouths. Furthermore, in males, absorption of tris-BP through the scrotum should be of particular concern since the human scrotum is about 20 times more permeable to chemicals than is other skin (11, 19).

When the CPSC banned the use of tris-BP in children's sleepwear in April 1977, washed garments were not recalled, because it was assumed that washing considerably reduces the risk of absorption of tris-BP. However, several lines of evidence suggest that tris-BP may still be absorbed from washed sleepwear and pose a risk to the wearer. (i) The flammability standards for children's sleepwear require that the garments still be flame resistant after 50 washings: in one study the total tris-BP in a fabric only decreased from 5.8 to 5.1 percent after 50 washes (7). (ii) A distinction has been made between tris-BP in the interior of the fiber and readily extractible "surface" tris-BP, much of which is removed by repeated washings (7, 9). In most cases, surface tris-BP is reduced more than 90 percent by three washings; in one case, five washings resulted in a 67 percent reduction of surface tris-BP (from 2.6 to 0.8 percent) (9). However, tris-BP is likely to continue diffusing from the inside of the fiber to the outside as the garment is being worn leading to continuing availability of the chemical for absorption through the skin or by mouth (11). (iii) A reduction of the amount of tris-BP available does not necessarily imply an equally reduced level of absorption. In studies in which tris-BP (0.9 ml/kg) was painted on the skin, 3.5 percent was absorbed over 96 hours; when the dose was

decreased to 0.05 ml/kg, the absorption increased markedly to 15.2 percent (18). (iv) Our results showing the presence of dibromopropanol in the urine of nine children wearing well-washed sleepwear suggests that tris-BP is available for absorption from well-washed sleepwear. However, an alternative explanation, which has not been ruled out, is that tris-BP was absorbed from the sleepwear when new and that the tris-BP or a metabolite, was stored in the body and then slowly excreted. Animal studies (18) indicate a relatively rapid rate of metabolism after gavage with tris-BP, but skin absorption and repeated exposure may result in deposition of tris-BP in fat stores, followed by slow hydrolysis and release of 2,3-dibromopropanol.

Children's garments are commonly worn by several children in a family and used for several years. Absorption of tris-BP from old, treated sleepwear could pose a continuing problem since about half of the children's sleepwear sold between July 1973 and April 1977 was treated with this chemical.

In addition to the hazard that tris-BP may cause cancer, absorption of this chemical may lead to heritable mutations in humans. Tris-BP is known to be a mutagen in *Salmonella* (1, 2), to damage DNA in vitro (20), to act as a potent mutagen in *Drosophila* (21), to cause unscheduled DNA synthesis in human cells in tissue culture (22), and to cause sister chromatid exchange and growth inhibition of Chinese hamster cells (23).

Potential adverse reproductive effects from tris-BP are also a concern. This chemical causes testicular atrophy and sterility in rabbits (8), and its metabolite 2,3-dibromopropanol is closely related in structure to 1,2-dibromo-3-chloropropane (DBCP), which has caused sterility in at least 80 male factory workers (24). Like tris-BP, DBCP is a mutagen in *Salmonella* (25), a carcinogen (26), and causes testicular atrophy and sterility in animals (27). 2,3-Dibromopropanol should itself be considered as a possible human mutagen and carcinogen.

Tris-BP, as well as structurally similar flame retardants such as Fyrol FR2 (28), may pose a grave threat to human health. Our study provides direct evidence that children in the United States exposed to tris-BP from their sleepwear have absorbed and stored this chemical in their bodies. Repeatedly washed tris-BP-treated sleepwear still contains large amounts of the chemical and is likely to pose a continuing hazard.

Testing of textile additives for deleterious long-term biological effects like mutagenicity, carcinogenicity, and tera-

togenicity is not required by law. It is very difficult to ascertain what chemical additives are present in a garment. Our study demonstrates the absorption of a biologically hazardous chemical from fabric into people. This absorption and the presence of a wide variety of additives in fabrics suggests the need for fabric labeling to identify additives, and toxicological testing before marketing.

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29. We thank Lois Gold, Robert H. Harris, Kim Hooper, Karen Kenyon, and Cedric Shakleton for their considerable help on the project. Supported by DOE contract EY-76-S-03-0034-PA156 to B.N.A.; NIH grants GM-13901 and HL-17269 and grant Q-125 of the Robert A. Welch Foundation to E.C.H.

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Phagocytosis in the Retinal Pigment Epithelium of the RCS Rat

Abstract. The retinal pigment epithelium of RCS rats, previously thought not to phagocytize photoreceptor outer segments, exhibited a peak of phagocytosis in vivo when animals were kept under conditions of cyclic lighting (12 hours of darkness and 12 hours of light). This peak occurred at 1 hour after the onset of light, with maximum and minimum levels of phagocytosis averaging about 5 percent of that found in the pigment epithelium of Osborn-Mendel rats used as a control. Eyecups that were obtained from Osborn-Mendel rats and maintained for up to 3 hours in organ culture demonstrated levels of phagocytosis that were sevenfold greater than those of unincubated controls. Likewise, a tenfold increase occurred in incubated as opposed to unincubated RCS eyes, raising the possibility that phagocytosis could be experimentally stimulated in vivo.

Although the retinal pigment epithelium of the RCS rat has been thought not to phagocytize the debris shed from rod outer segments (1), we now present evidence that such phagocytosis occurs, but at a greatly diminished rate relative to that of the normal rat. Furthermore, the apparent rate of phagocytosis in RCS pigment epithelium varies with a diurnal rhythm similar to that of unaffected animals (2), and the rate can be increased by maintaining the eyecup in organ culture.

Table 1. Number of phagosomes in pigment epithelium of OM and RCS rats. Each value represents the mean \pm standard error of the mean of phagosomes per 180- μ m field from six sections per eye for two eyes. In all experiments the lights were on between 0700 and 1900. The RCS peak at 0800 is significantly ($P < .005$, by one-way analysis of variance) different from the other levels.

| Time of enucleation | Large phagosomes per 180 μ m | |
|---------------------|----------------------------------|------------------|
| | OM | RCS |
| 0600 | 4.9 \pm 0.36 | 0.54 \pm 0.03 |
| 0630 | | 0.23 \pm 0.03 |
| 0700 | 4.4 \pm 1.7 | 0.49 \pm 0.14* |
| 0730 | 9.3 \pm 1.4 | 0.65 \pm 0.29* |
| 0800 | 29.0 \pm 5.2 | 1.58 \pm 0.23* |
| 0830 | 29.4 \pm 1.8 | 0.90 \pm 0.20* |
| 0900 | | 0.40 \pm 0.12* |
| 0930 | 12.0 \pm 8.0 | 0.52 \pm 0.16* |
| 1100 | 6.3 \pm 0.7 | 0.44 \pm 0.15 |

*Average of two separate experiments (four eyes).

Phagocytosis is an integral part of the process of photoreceptor renewal, in which new discs are added to the base of the rod outer segment and packets of old discs are shed at the apex (3). The shed discs are phagocytized by the retinal pigment epithelium and, if the animals are maintained under cyclic lighting (12 hours of light and 12 hours of darkness), the bulk of this shedding occurs in a peak within 2 hours of the beginning of the light phase of the cycle (2, 4). If the process of phagocytosis is defective, an imbalance can result between synthesis and degradation of outer segment membranes, leading to an accumulation of outer segment debris and eventual photoreceptor death. Such a defect occurs in the RCS rat (1), in which a genetic abnormality leads to the accumulation of rod outer segment debris from postnatal day 12 through 3 months of age, when few photoreceptor cells survive. No evidence has been reported of phagocytosis in the pigment epithelium of eyes of intact, affected animals (1), although the pigment epithelium of such animals can phagocytize carbon particles and, in rare cases, exogenous outer segment fragments which have been introduced subretinally (5). Since it has been demonstrated that the genetic lesion is in the pigment epithelium (6), cultures of RCS pigment epithelium were studied and