Rapid #: -22066207

CROSS REF ID: **242398**

LENDER: **INTARCHIVE (Internet Archive) :: Main Library**

BORROWER: **ULN (University of Mass. Lowell) :: OLeary Library**

TYPE:	Article CC:CCL
JOURNAL TITLE:	JAT. Journal of applied toxicology
USER JOURNAL TITLE:	Journal of Applied Toxicology
ARTICLE TITLE:	Comparative studies on nephrotoxic effects of Tris (2,3□dibromopropyl) phosphate and Bis (2,3□dibromopropyl) phosphate on rat urinary metabolites
ARTICLE AUTHOR:	Fukuoka, Masamichi,
VOLUME:	8
ISSUE:	1
MONTH:	
YEAR:	19880101
PAGES:	43-
ISSN:	0260-437X
OCLC #:	

Processed by RapidX: 2/15/2024 12:50:38 PM

Internet Archive Interlibrary Loan Fulfillment

Request on Thursday, February 15th, 2024
Journal of Applied Toxicology 1981-2015
0260-437X
Comparative studies on nephrotoxic effects of Tris (2,3-dibromopropyl) phosphate and Bis (2,3-dibromopropyl) phosphate on rat urinary metabolites
Fukuoka, Masamichi,
volume 8 issue 1 year 1988
pages 43-

Response from Internet Archive from: https://archive.org/details/sim_journal-of-applied-toxicology_1988-02_8_1
We hope this is helpful.

"I'm tracking your interlibrary loans ... ooo, the truck just hit a pothole in Poughkeepsie."

Did we make your day? Tweet us @internetarchive

Please explore the Internet Archive!

- Archive Scholar for fulltext search of over 25 million research articles
- https://archive.org/details/inlibrary and OpenLibrary.org for books to borrow
- Archive-it.org for library curated web collections
- Audio, software, TV News, and the Wayback Machine as well.

The Internet Archive is a non-profit library with a mission to provide Universal Access to All Knowledge.

info@archive.org

JOURNAL OF APPLIED TOXICOLOGY, VOL. 8(1), 43–52 (1988)

Comparative Studies on Nephrotoxic Effects of Tris (2,3-Dibromopropyl) Phosphate and Bis (2,3-Dibromopropyl) Phosphate on Rat Urinary Metabolites

Masamichi Fukuoka† and Terue Takahashi
Division of Medical Chemistry, National Institute of Hygienic Sciences, Kamiyoga-1-chome, Setagayaku, Tokyo 158, Japan

Katsushi Naito and Koichi Takada
Division of Toxicology, Biological Safety Research Center, National Institute of Hygienic Sciences

Key words: Tris (2,3,-dibromopropyl) phosphate; bis (2,3-dibromopropyl) phosphate; flame retardant; urinary metabolite; glucose; lactate; citrate; urinary enzymes; lactate dehydrogenase isoenzymes.

The mechanism of Tris-BP or Bis-BP (a metabolite of Tris-BP) induced nephrotoxicity was investigated by determining urinary excretion of enzymes and selected metabolites. Rats received single oral doses of 0, 71.7, 143.4 and 286.8 μmol/kg tris (2,3-dibromopropyl) phosphate (Tris-BP) or bis (2,3-dibromopropyl) phosphate (Bis-BP). Urine was collected over a 24 h period and subjected to biochemical examinations. Comparative studies on Tris-BP- and Bis-BP-induced nephrotoxicities were carried out for abnormal patterns of urinary excretion. The urinary excretion of glucose was higher in Bis-BP than Tris-BP at a dose of 143.4 μmol/kg, but this pattern reversed at a dose of 286.8 μmol/kg. Peak lactate excretion occurred later than peak glucose excretion with 143.4 and 286.8 μmol/kg Tris BP and 143.4 μmol/kg Bis-BP. Bis-BP 286.8 μmol/kg caused a transient urinary elevation of lactate on Day 2. Uric acid was excreted at higher levels for Bis-BP than Tris-BP on day 2 of urine collection. Activities of urinary enzymes including alkaline phosphatase, aspartate aminotransferase and γ-glutamyltransferase, were different on the first day of post-treatment for Tris-BP and Bis-BP. Leucine aminopeptidase and lactate dehydrogenase levels differed on the second day. Activities of the former enzymes on the day 2 urine suggested a transformation of Tris-BP to Bis-BP. Urinary patterns of lactate dehydrogenase isoenzymes (LDH-1–LDH-5) were different between Tris-BP and Bis-BP when rats were treated with the dose of 286.8 μmol/kg: Tris-BP caused a higher excretion of LDH-4 and LDH-5 in urine on day 1 and all five isoenzymes into the day 2 urine. Bis-BP caused slightly higher excretion of LDH-5 and LDH-4 into the day 1 and 3 urine, respectively. Bis-BP but not Tris-BP caused abnormally urinary excretion of sodium ion. Histopathologically, the nephrotoxic effect of Tris-BP appeared one day later and was more obvious than that of Bis-BP in rats after single oral administration.

INTRODUCTION

Tris (2,3-dibromopropyl) phosphate (Tris-BP) and Bis (2,3-dibromopropyl) phosphate (Bis-BP) are flame retardants that have been used extensively by the clothing industry. In 1977, Tris-BP-production was discontinued because of concerns that the chemical may be mutagenic and carcinogenic.[1,2] In 1981, Bis-BP, a metabolite of Tris-BP, was prohibited for use on household products such as carpets, sleepwear and mattresses in Japan.[3] Recently, Bis-BP, in equimolar doses, caused polyuria with higher elevation of the serum creatinine and greater depression of *para*-aminohippurate and *N*-methylnicotinamide uptakes than did Tris-BP on i.v. and i.p. administration to rats.[4,5] Therefore, Bis-BP may mediate the nephrotoxicity associated with Tris-BP.[4,5]

In a previous paper,[6] we reported that oral administration of 286.8 μmol/kg Tris-BP caused renal necrosis and high urinary excretion of lactate, glucose and enzymes. We also observed that Tris-BP caused a slight difference in the urinary excretion of citrate and uric acid.

This study was designed to clarify the mechanism of Tris-BP-induced nephrotoxicity in rats by determining urinary excretions of enzymes and metabolites compared with the toxic effect of Bis-BP, a metabolite of Tris-BP.

EXPERIMENTAL

Materials

Tris-BP and Bis-BP had a purity of 98% and were supplied by Daihachi Chemical Industries Ltd., Ohsaka, Japan. Citric acid, L-lactic acid and uric acid

†Author to whom correspondence should be addressed.

0260–437X/88/010043–10$05.00

Received 26 March 1987
Accepted 1 July 1987

reagents were 99.9% pure and purchased from Waka Pure Chemical Ind. Ltd., Tokyo, Japan.

Animal, dose, and sample collection

Since the nephrotoxic effect by Tris-BP was more obvious for male than female rats[2] and most investigations have been carried out on male rats,[4,5] male rats were used in our experiment. Adult male Wistar rats (weight range, 140–150 g) were obtained from Nippon Bio-supp. Center, Tokyo and were acclimated to laboratory conditions for 3 weeks. Rats (weight range, 220–230 g) were fasted for 18 h prior to use. Forty-eight rats were divided into two experiments, Exp. A (seven groups of three animals/group) and Exp. B (three groups of nine animals/group). Rats were given Tris-BP or Bis-BP at single oral doses of 0, 71.7, 143.4 and 286.8 μmol/kg BW (0 and 286.8 μmol/kg for Exp.B), in olive oil. In order to minimize the effect of circadian rhythm, dosing was done at the same time of day. Control rats were treated with olive oil (1.0 ml/rat) as a vehicle. The volume and pH of the 24 h urine samples were recorded for 7 days in Exp. A (but 3 days in the Exp.B). The urine samples were subjected to biochemical assays. All rats were kept in individual metabolic cages.

Biochemical assays

Creatinine in the blood, glucose and uric acid in the urine, and the activities of urinary enzymes including alanine aminopeptidase (GPT), alkaline phosphatase (ALP), aspartate aminotransferase (GOT), γ-glutamyl transferase (γ-GTP), leucine aminopeptidase (LAP), and lactate dehydrogenase (LDH), were determined by using commercially available assay kits on an Electro-Nucleonics centrifugal analyzer Model Gemsac IV, Fairfield, USA. Sodium and potassium were determined on a Hitachi Atomic Absorption Spectrophotometer Model 170–50 (Hitachi, Tokyo) at 589.5 and 765.8 nm, respectively. Lactic and citric acids were assayed according to the procedure of Barker and Summerson,[7] and by the McArdle modification of the pentabromoacetone method,[8] respectively. The amount of lactic and citric acids were calculated from calibration curves read on a Hitachi UV spectrometer Model 200–20 (Hitachi, Tokyo) at 560 and 500 nm, respectively. The curves were obtained from the pure acid (1, 5,10 and 20 μg) dissolved in water.

Kidney histopathology

Since Tris-BP was proved not to cause renal tubular necrosis and enzymuria at doses of 71.7 and 143.4 μmol/kg[6], histopathological examination was carried out only at a dose of 286.8 μmol/kg, in order to relate tubular necrosis with release of metabolic fuels and enzymes, including the LDH isoenzymes, from the kidney into the urine. Rats were killed by decapitation on days 1, 2, 3, and 7. The kidneys were weighed within 1 min of removal and the left kidney was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of the kidney were stained with hematoxylin–eosin, or with PAS and aldehyde–fuchsin as occasion demands. The right kidney was homogenized with 5 vol of 0.25 M sucrose and centrifuged at 10000 *g* for 10 min. The supernatant was subjected to the separation of LDH-isoenzymes as described below.

Separation of lactate dehydrogenase isoenzymes

The urine (1–2 μl) was loaded onto cellulose acetate plates (76 × 60 mm, Titan III-LIPO, Helena Laboratories, Beaumont, Texas) and the LDH isoenzymes were separated by electrophoresis in 0.05 M Tris–barbital buffer (pH 8.8) for 40 min at 4°C at a constant current of 5 mA/cm. Bands were made visible by incubating the plates at 37°C for 60 min with LDH isoenzymes test kit reagent (Helena Laboratories). Relative isoenzyme activity was obtained from the total LDH activity in the urine by using a Gelman DCD-16 densitometry (Gelman Sciences Inc., Michigan) at 570 nm. The total activity was determined by using an assay kit of LDH-UV Test (Wako Pure Chemical Industries Ltd., Tokyo).

Statistical analysis

Statistical analysis was carried out according to the guide.[9] Analyses of biochemical data were evaluated by using Bertlett's test for homogeneity of variances, analysis of variance (ANOVA) and Dunnett type mean rank test.

RESULTS

Urine volume

Tris-BP induced a greater degree of polyuria than did Bis-BP on Days 1, 2 and 3 at doses of 143.4 and 286.8 μmol, but Tris-BP induced a polyuria similar to Bis-BP at a dose of 71.7 μmol (Fig. 1). Bis-BP treated animals showed a trend of anuria from Day 4 at the dose of 286.8 μmol.

Biochemical examination

Levels of serum creatinine were 0.45 ± 0.4 mg/dl for control rats, 0.96 ± 0.15 mg/dl ($P < 0.05$) at 24 h, 1.17 ± 0.21 mg/dl ($P < 0.01$) at 48 h and 1.63 ± 0.30 mg/dl ($P < 0.01$) at 72 h for rats treated with Tris-BP at the dose of 286.8 μmol/kg, and 0.57 ± 0.08 mg/dl at 24 h, 0.53 ± 0.05 mg/dl at 48 h and 0.54 ± 0.09 mg/dl at 72 h for Bis-BP at 286.8 μmol/kg. Tris-BP did not cause an abnormal urinary excretion of potassium and sodium beyond the range of controls (2.64 ± 0.72 and 4.32 ± 0.70 meq/day, respectively). However, Bis-BP caused an excretion of K (6.07 ± 0.62 meq/day) and a 3.8-fold increase in excretion of Na (16.42 ± 0.75 meq/day) ($P < 0.05$) on Day 2. Tris- and Bis-BP did not significantly elevate urinary levels of citrate beyond those of the control (7.9 ± 1.3 mg/day).

The excretions of lactate, uric acid, glucose and enzymes, ALP, GOT, GPT, γ-GTP, LAP and LDH, were significantly elevated in the urine of rats treated with Tris-BP or Bis-BP (Figs. 2–10). Urinary levels of

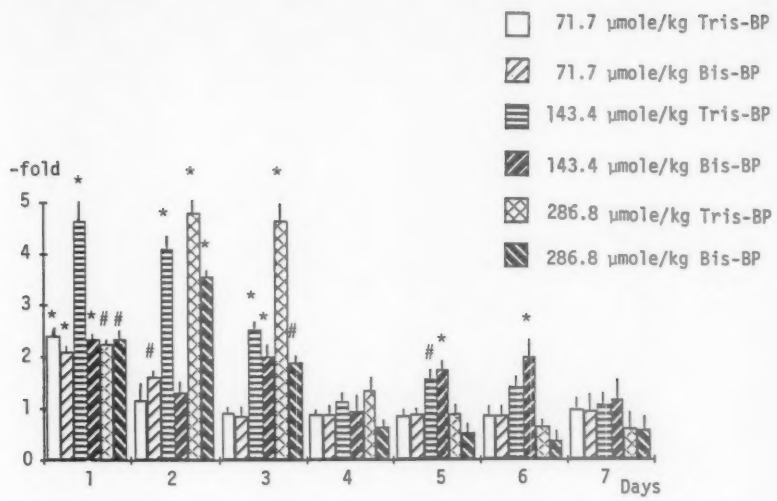

Figure 1. Urine volumes of rats at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Urine volumes of the control rats are obtained from three rats for 7 days and a mean volume is 17.8 ± 1.31 (S.D.) ml/day. [b]Results show a multifold increase in excretory volume over the control mean values. [c]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

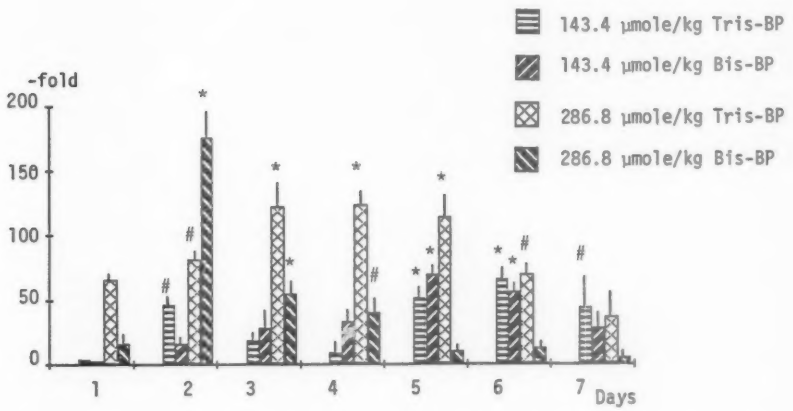

Figure 2. Urinary levels of lactate at various times after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean levels of urinary lactate were obtained from three control rats for 7 days: 1.5 ± 0.3 (S.D.) mg/day. [b]Since the dose of 71.7 μmol/kg did not cause abnormal urinary levels of lactate beyond the range of the controls, the results are eliminated. [c]Results show a multifold increase in excretory lactate over the control mean values. [d]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

lactate and uric acid showed no significant difference between controls and rats treated with a dose of 71.7 μmol/kg. At a dose of 143.4 μmol/kg, Tris-BP gave a peak lactate excretion on Day 6, 1 day later than Bis-BP. At a dose of 286.8 μmol/kg, Tris-BP caused an abnormally prolonged excretion of lactate but Bis-BP gave a transient peak on Day 2. Both Tris- and Bis-BP gave two peaks of uric acid excretion; Day 2 and Day 5 or 6 (Fig. 3). Glucose excretion was greater with Bis-BP than with Tris-BP at a dose of 143.4 μmol/kg but reversed at 286.8 μmol/kg (Fig. 4).

Transient urinary excretion of ALP, γ-GTP, GPT, GOT, LAP, and LDH were greater for Tris-BP than for Bis-BP at the dose of 286.8 μmol/kg (Figs. 5–10). Tris-BP treatment differed from Bis-BP in the urinary excretion patterns and activities of ALP, γ-GTP, GOT,

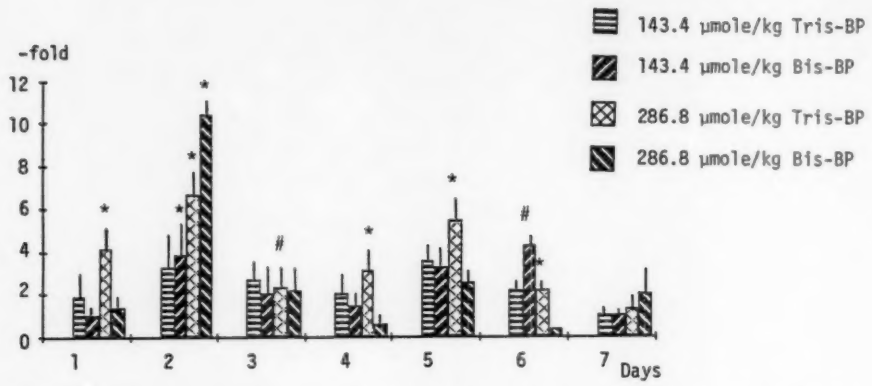

Figure 3. Urinary levels of uric acid at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean levels of urinary uric acid were obtained from three control rats for 7 days: 1.3 ± 0.2 (S.D.) mg/day. [b]Since the dose of 71.7 μmol/kg did not cause abnormal urinary levels of uric acid beyond the range of the controls, the results are eliminated. [c]A kit used for uric acid assay is Boehringer Mannheim Uric acid-uricase test. [d]Results show a multifold increase in excretory uric acid over the control mean values. [e]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

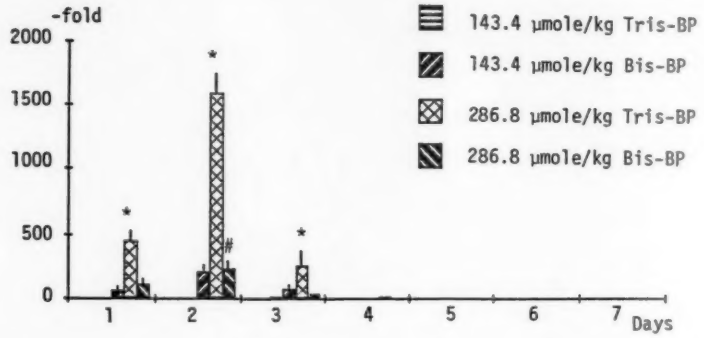

Figure 4. Urinary levels of glucose at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean levels of urinary glucose were obtained from three control rats for 7 days: 1.9 ± 0.6 (±S.D.) mg/day. [b]Since the dose of 71.7 μmol/kg did not cause abnormal urinary levels of glucose beyond the range of the controls, the results are eliminated. [c]A kit used for glucose assay is Boehringer Mannheim Autopack A glucose. [d]Results show a multifold increase in excretory glucose over the control mean values. [e]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

LAP and LDH on Day 1. Tris-BP treatment was similar to Bis-BP in the activities of ALP and γ-GTP on Day 2. GOT activity due to Tris-BP on Day 2 corresponded to that due to Bis-BP on Day 1. LAP and LDH activities due to Tris-BP at the dose of 286.8 μmol/kg corresponded to those due to Bis-BP at a dose of 573.6 μmol/kg (unpublished observation).

Kidney histopathology

The results are shown in Table 1: On Day 1 Tris-BP caused an acute tubular lesion characterized by pyknosis of the tubular epithelial cells in the outer medulla, but not in the cortex (Fig. 11). Severe tubular necrosis and desquamation were observed in 2/3 rats on day 2 (Fig. 12). On Day 3 severe tubular necrosis, desquamation and cells with large nuclei were noted in all rats. Regeneration of the cells in 1/3 rats was also noted (Fig. 13). On Day 2 Bis-BP produced less necrosis and desquamation of the tubular epithelial cells than did Tris-BP.

LDH-isoenzyme pattern

Results of LDH-isoenzyme activities are shown in Tables 2 and 3. Tris-BP slightly reduced LDH-5 activity in the kidney, significantly elevated the urinary excretion of LDH-4 and LDH-5 on Day 1, and caused the highest excretion of the five LDH isoenzymes on Day 2. Bis-BP caused an elevated excretion of LDH-5 on Day 1.

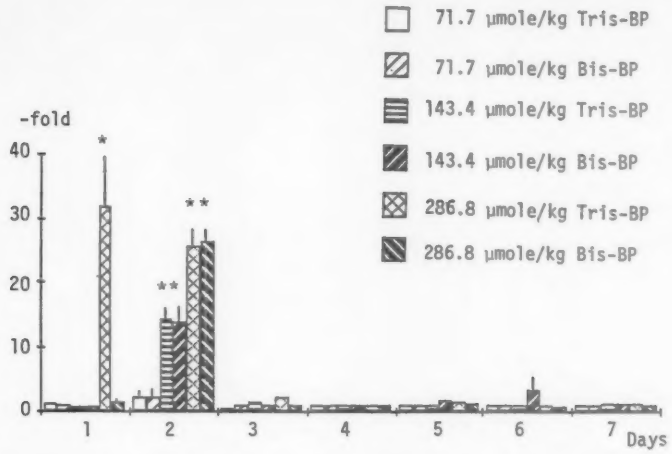

Figure 5. Urinary activity of ALP at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean activities of urinary ALP were obtained from three control rats for 7 days: 3259.2 ± 1120.3 (S.D.) mU/day. [b]A kit used for ALP assay is Wako Alkaline phosphatase B-AR II. [c]Results show a multifold increase in excretory ALP over the control mean values. [d]*: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

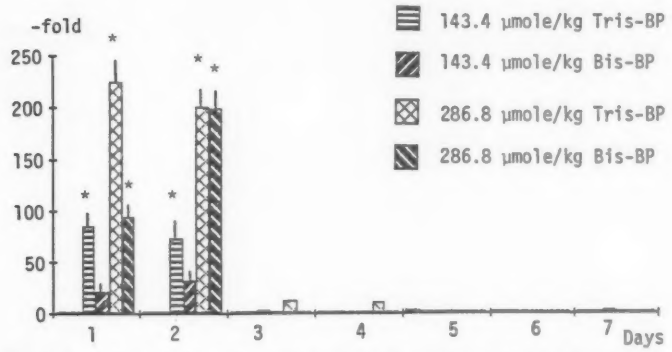

Figure 6. Urinary activity of γ-GTP at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean activities of urinary γ-GTP were obtained from three control rats for 7 days: 7488.2 ± 3614.4 (S.D.) mU/day. [b]Since the dose of 71.7 μmol/kg did not cause abnormal urinary activities of γ-GTP beyond the range of the controls, the results are eliminated. [c]A kit used for γ-GTP assay is Boehringer Mannheim γ-GT. [d]Results show a multifold increase in excretory γ-GTP over the control mean values. [e]*: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

DISCUSSION

Administration of Tris-BP to rats is known to cause proximal tubular damage and acute renal failure with elevation of the serum creatinine and urea and with depression of organic anion and cation transport.[4,6] It is known[10] that reabsorption of glucose and organic anions such as citrate and lactate is maintained across the brush border membrane by the sodium cotransport system and recent studies demonstrate that sodium urate is extensively reabsorbed in the proximal tubule.[11] In acute tubular nephropathy induced by toxic or ischemic injury, the prevailing theory[12,13] states that excretion of cytoplasmic enzymes indicates a low damage characterized by increased membrane permeability. Excretion of significant levels of organelle-linked enzymes indicates a severe damage accounting for the necrosis of the nephronic structure. In the present experiments, both Tris-BP and Bis-BP caused the excretion of metabolites and enzymes related to renal tubular necrosis and desquamation. However, there were some differences in the nephrotoxicity produced. Tris-BP caused a more obvious histopathological tubular lesion with more severe necrosis and desquamation in the tubular cells of the outer medullar on Day 3; 1 day later than Bis-BP. Also, the most severe changes induced by Tris-BP were noted 1 day later than the peak urinary excretion of glucose, uric acid and all the enzymes examined. On the other

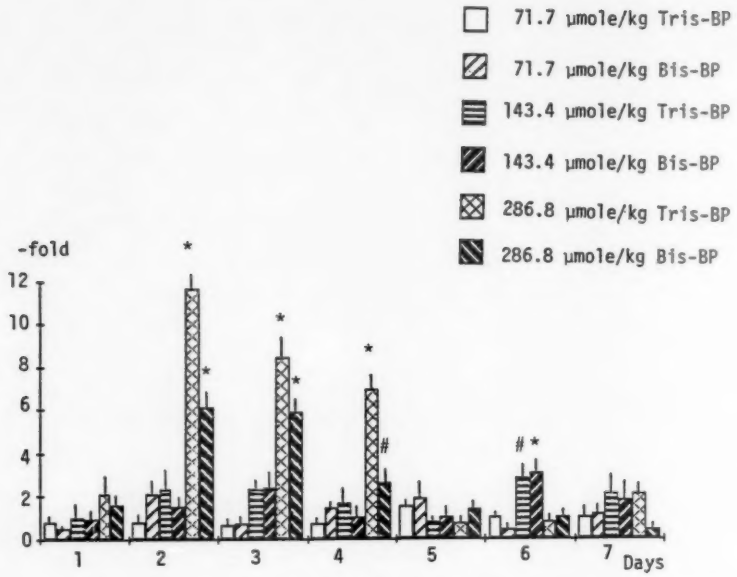

Figure 7. Urinary activity of GPT at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean activities of urinary GPT were obtained from three control rats for 7 days: 1360.8 ± 162.4 (S.D.) mU/day. [b]A kit used for GPT assay is Smithkleine S-GPT.[c]Results show a multifold increase in excretory GPT over the control mean values. [d]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

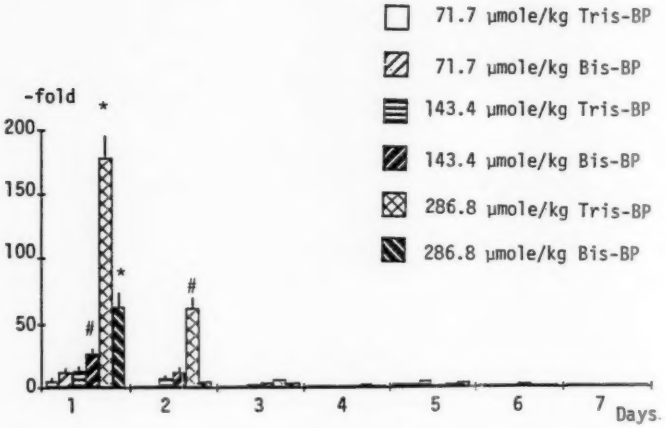

Figure 8. Urinary activity of GOT at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean activities of urinary GOT were obtained from three control rats for 7 days: 645.6 ± 134.4 (S.D.) mU/day. [b]A kit used for GOT assay is Smithkleine S-GOT. [c]Results show a multifold increase in excretory GOT over the control mean values. [d]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

hand, Bis-BP caused histopathological changes which coincided with the excretion peaks (except for GOT) on Day 2. Both Tris-BP and Bis-BP produced similar excretion patterns of uric acid and GPT (cytoplasmic enzyme), but different patterns for lactate, glucose, ALP, γ-GTP, GOT, LAP and LDH. The excretory patterns and activities of the organelle-linked enzymes like ALP, γ-GTP and GOT suggest that the nephrotoxic effect of Tris-BP might be metabolized to Bis-BP mediating the nephrotoxicity associated with Tris-BP from Day 2.

It is known that the major energy-producing process in the kidney is provided primarily by oxidation of metabolic fuels by the tubular cells.[14–16] Citrate, glucose

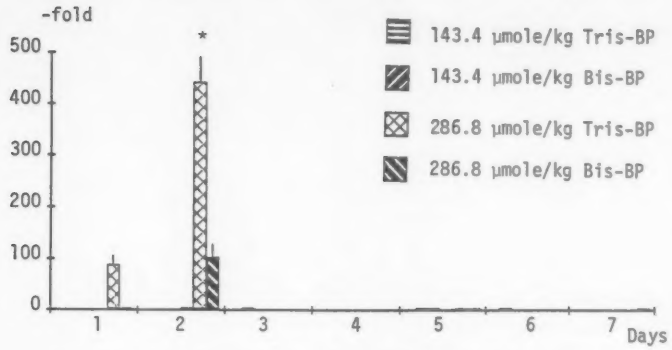

Figure 9. Urinary activity of LAP at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean activities of urinary LAP were obtained from three control rats for 7 days: 379.2 ± 166.6 (S.D.) mU/day. [b]Since the dose of 71.7 μmol/kg did not cause abnormal urinary activities of LAP beyond the range of the controls, the results are eliminated. [c]A kit used for LAP assay is Wako LAP-AR II. [d]Results show a multifold increase in excretory LAP over the control mean values. [e]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

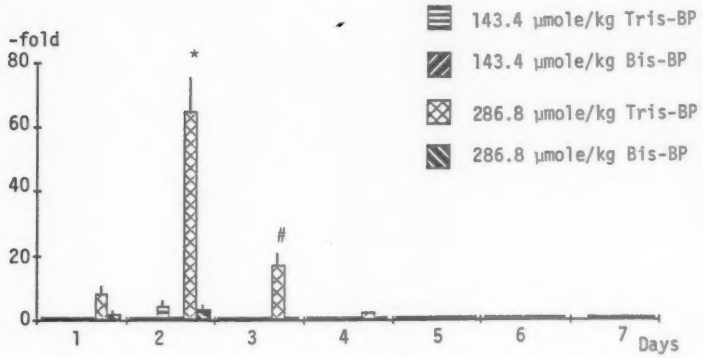

Figure 10. Urinary activity of LDH at various time after single dosing with Tris-BP and Bis-BP at 71.7, 143.4 and 286.8 μmol/kg, respectively. [a]Control mean activities of urinary LDH were obtained from three control rats for 7 days: 1938.7 ± 754.8 (S.D.) mU/day. [b]Since the dose of 71.7 μmol/kg did not cause abnormal urinary activities of LDH beyond the range of the controls, the results are eliminated. [c]A kit used for LDH assay is Wako LDH-UV-Test. [d]Results show a multifold increase in excretory LDH over the control mean values. [e]#: $P < 0.05$ and *: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

and lactate contribute approximately 10, 20 and 30%, respectively, to the fuel of the kidney.[16] Citrate also plays an important role by preventing precipitation of calcium in urine and kidney tissue.[17] Since we found that urinary output of citrate is undisturbed, Tris-BP or Bis-BP might not have a direct effect on the hypercalciuria normally associated with proximal tubular necrosis.[18]

Lactate is metabolized to pyruvate by LDH and eventually to glucose in the kidney. This lactate metabolism may be impaired by enzymatic defects, tissue hypoxia or ischemia.[19] The urinary excretion of lactate was observed as a progressive increased delay from the excretion peak of glucose at the doses of 143.4 and 286.8 μmol/kg Tris-BP and of 143.4 μmol/kg Bis-BP. However Bis-BP (286.8 μmol/kg) gave a transient peak on Day 2, coinciding with the peak glucose excretion. This difference may be due to changes in the LDH isoenzymes. In mercurial intoxication, significant changes of the LDH isoenzyme pattern have been reported in the rat. Mercury caused an increase of LDH-5 fraction in the cortical zone[20,21] and of the LDH-1 and -2 in the inner medulla[20] of rat kidney with acute tubular necrosis, indicating decreased oxygen consumption.[22] Mercury also caused a high urinary excretion of the LDH-1, -2 and -5 fractions.[20] Tris-BP but not Bis-BP produced both of these effects: a loss of the LDH-5 fraction from the kidney on Day 1 and a high urinary excretion of the LDH-1 (from Day 2), -4 and -5 fraction from Day 1 to Day 3.

Our data show that Tris-BP and Bis-BP might attack the energy-producing system in the kidney by somewhat different mechanisms. Tris-BP may exert a direct nephrotoxic effect on Day 1 or 2, partially followed by the action of Bis-BP. The necrotic lesion would explain the high urinary levels of cytosol and organelle-

Table 1. Incidence of histological findings of male rats administered Tris- and

Chemicals	Vehicle				Tris-BP		
Days	1st[a]	2nd[a]	3rd[a]	7th[b]	1st[a]	2nd[a]	3rd[a]
No. of animals examined	3	3	3	3	3	3	3
Body wt. (g)	215.3±12.9	234.0±11.4	232.0±14.2	301.6±13.2	212.7±7.3	218.7±17.9	233.3
Kidney wt. (g)	1.76±0.19	1.85±0.14	1.92±0.05	2.30±0.04	1.85±0.15	2.01±0.07	2.32±
Kidney/Body $\times 10^{-3}$	(8.25±0.55)	(8.17±0.34)	(8.08±1.69)	(7.75±0.22)	(8.72±0.77)	(9.23±0.44)	(10.0
Findings of left kidney (Tubuli)							
epithelial cast	±(1)	—	—	—	—	—	—
pyknosis	—	—	—	—	++(3)	+(3)	—
necrosis	—	—	—	—	—	++(1),+++(2)	+++
desquamation	—	—	—	—	—	++(1),+++(2)	+++
swelling	—	—	—	—	±(2)	±(1),+(2)	++(2
large nuclei	—	—	—	—	—	—	+(3)
hyaline cast	—	—	—	—	—	—	+++
regeneration	—	—	—	—	—	—	+(1),
dilatation	—	—	—	—	—	±(3)	+(3)

[a]– negative; ± slight; + mild; ++ marked; +++ severe, the number of animals
[b] were obtained from rats in Exp. A.

Table 2. LDH-Isoenzyme activity in the urine of rats administered Tris- and B

Days	Chemicals	Urine vol. ml	Total activity	LDH-1	LDH-2
1	Vehicle	8.2±1.9	1996.3±739.6	577.9±213.5	265.8±77.9
1	Tris-BP	6.7±3.0	11873.7±2204.1**	650.2±466.7	399.9±336.0
1	Bis-BP	10.2±0.6	5789.4±1015.5*	550.3±184.0	281.5±42.8
2	Vehicle	9.0±2.1	2064.0±842.6	639.8±304.8	286.7±118.4
2	Tris-BP	43.2±1.3	154643.4±9948.5*	48292.4±9513.2*	23374.6±57
2	Bis-BP	17.8±1.1	3284.2±796.4	1153.7±184.3	424.8±199.9
3	Vehicle	8.3±2.4	1641.0±200.0	372.3±35.3	209.1±130.5
3	Tris-BP	39.5±5.1	24590.3±9907.5*	7145.3±2817.8**	1292.6±967.
3	Bis-BP	17.8±6.2	2467.9±797.7	638.9±240.2	142.0±10.4

*: $P < 0.05$ and **: $P < 0.01$ compared with its respective control, Dunnett type

nd Bis-(2,3-dibromopropyl)phosphate

		Bis-BP			
rd[a]	7th[b]	1st[a]	2nd[a]	3rd[a]	7th[b]
	3	3	3	3	3
33.3±21.1	278.5±11.1	221.3±16.0	232.0±17.7	244.0±28.6	282.1±10.2
.32±0.05	2.63±0.17	1.77±0.07	1.85±0.13	1.94±0.09	2.40±0.29
10.04±0.95)	(9.45±0.42)	(8.04±0.49)	(7.97±0.41)	(7.94±0.79)	(8.38±0.79)
—	—	±(1)	—	—	±(1)
—	—	±(3)	+(2),++(1)	±(1),+(2)	—
++(3)	—	—	+(1),++(1)	—	—
++(3)	±(3)	—	+(2)	+(2)	±(2)
+(2)	—	—	±(1)	±(2),+(1)	—
+(3)	—	—	—	—	—
++(2)	—	—	—	—	—
+(1),++(1)	++(3)	—	—	—	+(2),++(1)
+(3)	—	—	—	—	—

als observed is given in parentheses.

d Bis-(2,3-dibromopropyl)phosphate

	LDH activities (mU) in Urine		
	LDH-3	LDH-4	LDH-5
7.9	176.2±73.8	250.7±104.5	725±279.0
36.0	103.6±42.3	1987.9±477.7**	8762.3±1522.2**
2.8	164.4±54.7	786.0±116.3	3984.9±450.5**
18.4	176.6±56.5	260.3±99.9	707.5±273.7
±5786.4*	11149.4±5736.1	13480.9±2276.8*	58346±10671.0*
99.9	122.6±40.3	291.6±106.8	1291.6±408.6
30.5	130.9±39.5	277.3±110.2	651.6±231.6
867.9	586.2±315.1	5263.0±1935.7**	10303.1±4241.0*
0.4	90.4±29.8	1093.4±387.2*	503.2±188.6

ype mean rank test.[9]

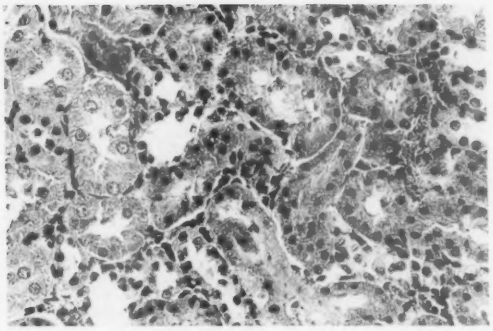

Figure 11. Left kidney of a rat killed at 24 h after a single dose (286.8 μmol/kg) with Tris-BP. Pyknosis of the tubular epithelium are extensive. (H & E, × 200)

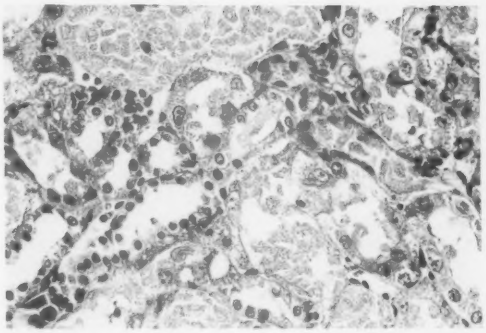

Figure 13. Left kidney of a rat killed at 72 h after a single dose (286.8 μmol/kg) with Tris-BP. Necrosis and desquamation of the tubular epithelium are very extensive, dilatation of the tubular lumen is extensive and large dysplasia nucleus are evident. (H & E, × 200)

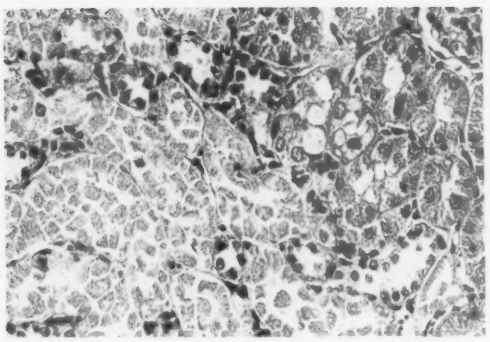

Figure 12. Left kidney of a rat killed at 48 h after a single dose (286.8 μmol/kg) with Tris-BP. Necrosis and desquamation of the tubular epithelium are extensive. (H & E, × 200)

linked enzymes. Also, the high urinary levels of glucose, lactate and uric acid suggest reduced reabsorption in the proximal tubule. The nephrotoxic mechanisms between Tris-BP and Bis-BP at the oral dose of 286.8 μmol/kg may be due to differences in absorption from the gastrointestinal lumen. Further work is in progress to investigate the absorption and metabolism of Tris-BP and Bis-BP.

Acknowledgement

The authors are grateful to Dr. Tsutomu Yamaha, at the Japan Health Sciences Foundation and Dr. Akira Tanaka, Division Chief of Biological Chemistry and Reference Standards, for their valuable advices, and Dr. Masuo Tobe, Division Chief of Toxicology and Dr. Kazuo Kobayashi and Dr. Yukio Nakaji, Division of Toxicology, for their keen interests during the course of this work, at the National Institute of Hygienic Sciences. The authors are also grateful to Miss Chiyuri Sato for the expert technical assistance.

Table 3. LDH isoenzyme activity of the right kidney from rats administered Tris- and Bis-(2,3-dibromopropyl)phosphate

Days	Chemicals	Body weight (g)	Kidney weight (g) (Kidney wt/Body wt × 10^{-3})		LDH-activity (mU) of the right kidney: Total LDH	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
1	Vehicle	215.3±12.9	1.76±0.19	(8.25±0.55)	150.8±48.4	42.8±12.7	19.0±14.1	8.2±4.9	31.3±9.7	49.5±5.3
	Tris-BP	212.7±7.3	1.85±0.15	(8.72±0.77)	125.0±8.0	32.5±2.2	24.3±1.9	8.8±1.5	25.6±4.1	33.8±3.0*
	Bis-BP	221.3±16.0	1.77±0.07	(8.04±0.49)	189.8±29.8	49.6±5.8	38.5±5.8	14.2±4.2	33.6±6.1	53.8±8.8
2	Vehicle	234.0±11.4	1.85±0.14	(8.17±0.34)	200.7±37.3	56.7±13.5	32.5±7.2	14.4±7.2	39.3±10.9	57.7±18.8
	Tris-BP	218.7±17.9	2.01±0.07	(9.23±0.44)*	175.4±36.1	38.8±9.3	36.5±6.1	19.7±3.9	34.4±6.6	46.0±12.1
	Bis-BP	232.0±17.7	1.85±0.13	(7.97±0.41)	161.7±37.2	44.3±7.9	38.5±8.1	13.7±2.6	23.8±1.9	41.1±12.6
3	Vehicle	232.0±14.2	1.92±0.05	(8.08±1.69)	178.9±46.4	55.1±16.3	24.6±7.0	13.2±4.5	35.9±10.2	50.1±15.9
	Tris-BP	233.3±21.1	2.32±0.05*	(10.04±0.95)*	376.7±53.4	89.0±12.9	80.2±10.1*	49.0±10.1**	66.7±10.8*	91.8±14.3
	Bis-BP	244.0±28.6	1.94±0.09	(7.94±0.79)	356.1±98.9	92.6±30.3	74.5±27.3*	33.7±10.9	65.1±12.0	90.1±22.8

*: $P < 0.05$ and **: $P < 0.01$ compared with its respective control, Dunnett type mean rank test.[9]

REFERENCES

1. Consumer Product Safety Commission, Children's wearing apparel containing TRIS; interpretation as banned hazardous substance. *Fed. Reg.* **42**, 18850–18954 (1977).
2. C. Reznik, J. M. Ward, J. F. Hardistry and A. Russfield, Renal carcinogenic and nephrotoxic effects of the flame retardant Tris(2,3-dibromopropyl)phosphate in F344 rats and (C57BL/6N × C3H/HeN) F_1 mice. *J. Natl. Cancer Inst.* **63**, 205–212 (1979).
3. Japanese Cabinet Order No. 257, July 27th, 1981.
4. W. C. Elliott, R. K. Lynn, D. C. Houghton, J. M. Kennish and W. M. Bennett, Nephrotoxicity of the flame retardant, Tris(2,3-dibromopropyl)phosphate, and its metabolites. *Toxicol. Appl. Pharmac.* **62**, 179–182 (1982).
5. R. K. Lynn, C. G-Could, K. Wong and J. M. Kennish, Metabolism, distribution, and excretion of the flame retardant, Tris (2,3-dibromopropyl) phosphate (Tris-BP) in rats: identification of mutagenic and nephrotoxic metabolites. *Toxicol. Appl. Pharmac.* **63**, 105–119 (1982).
6. M. Fukuoka, T. Takahashi, A. Tanaka, T. Yamaha, K. Naito, Y. Nakaji, K. Kobayashi and M. Tobe, Nephrotoxic effect of Tris (2,3-dibromopropyl) phosphate on rat urinary metabolites: Assessment from ^{13}carbon NMR spectra of urines, and biochemical and histopathological examinations. *J. Appl. Toxicol.*, in press.
7. S. B. Barker and W. H. Summerson, The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.* **138**, 535–554 (1941).
8. B. McArdle, A modified method for the microdetermination of citric acid. *Biochem. J.*, **60**, 647–649 (1955).
9. S. C. Gad and C. S. Weil, Statistics for toxicologists. In *Principles and Methods of Toxicology*, ed. by A. W. Hayes, pp. 273–320, Raven Press, New York (1982).
10. R. Kinne, Transport function of renal cell membranes: Sodium cotransport systems. In *Renal Biochemistry, Cells, Membranes, Molecules* ed. by R. K. H. Kinne, pp. 99–141, Elsevier, Amsterdam, New York, Oxford (1985).
11. D. W. Martin, Metabolism of purine and pyrimidine nucleotide. In *Harper's Review of Biochemistry* ed. by D. W. Martin Jr, P. A. Mayers, V. W. Rodwell and D.K. Granner, 20th ed, pp. 357–375, Maruzen Asian Edition, Lange Medical Publications, Maruzen, (1985/1986).
12. G. Emanuelli, G. Calcamuggi, G. Cestonaro, G. Gatti, G. Anfossi and A. Cotto, Urinary enzyme activities in human and experimental renal damage. *Excerpta Med. Int. Cong. Ser.* **502**, 780–781 (1979).
13. K. Nomiyama, A. Yamamoto and C. Sato, Assay of urinary enzymes in toxic nephropathy. *Toxicol. Appl. Pharmac.* **27**, 484–490 (1974).
14. K. Kurokawa, G. T. Nagami and D. T. Yamaguchi, Transport and substrate metabolism of the kidney. In *Renal Biochemistry, Cells, Membranes, Molecules* ed. by R. K. H. Kinne, pp.175–223, Elsevier, Amsterdam, New York, Oxford (1985).
15. E. Leal-Pinto, H. C. Park, F. King, M. D. MacLeod and R. F. Pitts, Metabolism of lactate by the intact functioning kidney of the dog. *Am. J. Physiol.* **224**, 1463–1467 (1973).
16. R. F. Pitts, Production of CO_2 by the intact functioning kidney of the dog. *Med. Clin. N. Am.* **59**, 507–518 (1975).
17. J. J. Cohen and D. E. Kamm, Renal metabolism in relation to renal function. In *The Kidney*, ed by B. M. Brenner and R. C. Rector, 2nd ed. pp. 144–248, W. B. Saunder & Co., Philadelphia (1981).
18. E. L. Foulkes, Nature of inhibition of renal aspartate reabsorption in experimental Fanconi syndrome. *Toxicol. Appl. Pharmac.* **71**, 445–450 (1983).
19. M. G. Cogan, F. C. Rector Jr and D. W. Seldin, Acid-base disorders. In *The Kidney*, ed. by B. M. Brenner and R. C. Rector, 2nd ed. pp. 841–907, W. B. Saunder & Co., Philadelphia (1981).
20. S. Ringoir, LDH isoenzyme pattern in rat kidney in mercurial intoxication. *Nephron.* **7**, 538–544 (1970).
21. G. Emanuelli, G. Cestanaro, G. Anfossi, G. Calcamuggi, G. Gatti and C. Marcarino, Urinary enzyme excretion and renal lactate dehydrogenase isoenzyme pattern in acute mercuric chloride nephropathy of rat. *Enzyme* **27**, 89–98 (1982).
22. H.L.Conn, L. Wilds and J. Helwig, A study of the renal circulation, tubular function and morphology, and urinary volume and composition in dogs following mercury poisoning and transfusion of human blood. *J. Clin. Invest.* **33**, 732–741 (1954).