[Chem. Pharm. Bull. 16(9)1655—1660(1968)]

UDC 615.917.015.36 : 547.584

Toxicity of Terephthalic Acid

Akio Hoshi, Reiko Yanai, and Kazuo Kuretani

Pharmacology Division, National Cancer Center Research Institute[1]

(Received August 16, 1967)

The acute toxicity of terephthalic acid and its sodium salt, the effect of terephthalic acid administration on liver and renal functions, and the chemical composition of blood plasma were studied.

LD_{50} of terephthalic acid in mice was found to be more than 5000 mg/kg by oral administration and 1430 mg/kg by intraperitoneal injection. Those of its sodium salt (Na_2TPA) were 6300 mg/kg (oral), 8600 mg/kg (subcutaneous), 4600 mg/kg (intraperitoneal), and more than 1300 mg/kg (intravenous).

Terephthalic acid feeding for 7 days (0.5% in diet) did not affect the dye (PSP) excretion from kidney, the transaminase activity (GOT and GPT) in blood plasma, and the contents of sugar, protein, free amino acids, and urea in blood plasma. The BSP retention in liver was not increased, but rather decreased. The barbiturate sleeping-time was shortened markedly by terephthalic acid feeding.

In view of these facts, it was concluded that terephthaic acid did not show any toxic indications in mice fed 0.5% terephthalic acid diet.

Terephthalic acid has a potentiating effect on biologically active substances such as tetracycline-type antibiotics,[2] thiamine,[3] and sulfonamides.[4] In the previous studies on the distribution of terephthalic acid in body and its excretion in urine, a very interesting fact was observed that the terephthalic acid content in tissues was maintained at low level, even though higher content was found in liver and kidney than in plasma.[5] Furthermore, it was found not to be metabolized but to be rapidly excreted, almost quantitatively in urine as an intact form.[6] In the terephthalic acid feeding experiment for a long period, animals were not affected suppressively in either growing, maturing, or breeding.[7] Judging from these results, it is supposed that terephthalic acid would be biologically active and nontoxic.

The purpose of the present studies is to investigate the acute toxicity of terephthalic acid and its sodium salt, and also the effect of administration towards physiological functions such as liver and renal functions.

Materials and Methods

Animals——Animals used were female Swiss albino mice weighing 25±3 g and female Wistar King-A rats of 200±20 g. Treatment groups for feeding experiments were fed with the diet containing 0.5% terephthalic acid in a commercial diet for 7 days. Other animals were fed with a commercial chow diet (CA-1: CLEA Japan, Inc., Tokyo). Diet and water were given *ad libitum*.

1) Location: *Tsukiji 5-Chome, Chuo-ku, Tokyo.*
2) R.E. Elliot, "American Cyanamid Company 10th Animal Feed Symposium," 15 (1959); E.H. Peterson, W.L. Hendrix, and L.D. Braddy, *Poultry Sci.*, **38**, 235 (1959); K.E. Price and Z. Zolli, Jr., *Avian Disearses*, **3**, 157 (1959).
3) K. Kuretani, A. Hoshi, and Y. Hirayama, *Nippon Chikusan Gakkai-ho*, **36**, 511 (1965); A. Hoshi, R. Yanai, and K. Kuretani, *ibid.*, **38**, 1 (1967).
4) A. Hoshi, R. Yanai, and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **15**, 1138 (1967); K. Takagi, T. Suzuki, and Y. Saitoh, *ibid.*, **15**, 1597 (1967).
5) A. Hoshi and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **16**, 131 (1968).
6) A. Hoshi and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **15**, 1978 (1967).
7) H. Nagasawa and K. Kuretani, *Nippon Chikusan Gakkai-ho*, **36**, 392 (1965).

Chemicals——Terephthalic acid was of 99.7% purity (Teijin Ltd.) and its sodium salt was prepared by dissolving 100 g of terephthalic acid in 750 ml of 2N NaOH solution, filtered and added with 3 liters of 99% EtOH. The precipitated sodium salt was collected by filtration, washed several times with EtOH on Büchner funnel, and dried at 60°. Phenolsulfophthalein (Wako Pure Chemical Ind., Ltd.), a reagent kit for transaminase (Chugai Pharmaceutical Co., Ltd.), bromosulphalein (Diichi Pure Chemicals Co., Ltd.), pentobarbital sodium (Abbott Laboratories: Nembutal Sodium), and Probenecid (Nippon Merck–Banyu Co., Ltd.:Probenemid) were used. Other chemicals used for analysis were of analytical grade.

Acute Toxicity Test——The acute toxicity of terephthalic acid and its sodium salt was examined through various administration routes. Thirty mice were used in each experiment. Terephthalic acid suspensions were prepared in a concentration of 10 and 20% of the acid in a 0.5% sodium carboxymethylcellulose solution. These suspensions were used for oral and intraperitoneal administrations. Sodium salt of the acid was suspended in distilled water to 25.2% corresponding to 20% terephthalic acid, which was used for oral, intraperitoneal, and subcutaneous administrations. For intravenous administration, 12.6% solution, equal to 10% of terephthalic acid, was used. Only in oral administration, mice were starved for 6 hours before use. The animals were kept 5 to a cage ($18 \times 30 \times 12$ cm) during the experiments in a room maintained at $24 \pm 1°$ and $55 \pm 5\%$ relative humidity. Three days after administration, a number of dead mice was counted, and LD_{50} was calculated by the Litchfield–Wilcoxon method.[8]

Renal Excretion Test——Phenolsulfophthalein was used for the excretion test and its retention was determined by the Scarborough method.[9] Blood sample was collected from *vena cava* 30 min after intravenous administration of 75 mg/kg PSP.

Transaminase Activity in Blood Plasma——The activity of the transaminases (glutamic–oxaloacetic transaminase=GOT, and glutamic–pyruvic transaminase=GPT) in blood plasma were determined by the Reitman–Frankel method.[10]

Bromosulphalein Retention Test——The retention rate of bromosulphalein (BSP) was determined by the Kutob method.[11]

Barbiturate Sleeping-time Method——The barbiturate sleeping-time was determined by the Kutob method,[12] except that the dose of pentobarbital sodium was lowered to 40 mg/kg instead of 45 mg/kg, because Swiss albino mouse is sensitive to this drug.

Chemical Composition of Blood Plasma——Determination of the content of sugar, protein, free amino acid–nitrogen, and urea–nitrogen in plasma was carried out by the respective methods of Scott[13] with anthrone reagent, Gornall[14] with biuret reagent, Spies[15] with $CuCl_2$ reagent, and Fister[16] with diacetyl monoxime, and the respective standard compounds were glucose, bovine albumine, L–alanine, and urea.

Results

Acute Toxicity of Terephthalic Acid and Its Sodium Salt

The acute toxicity of terephthalic acid through various administration routes was determined. As shown in Table I, LD_{50} of the acid was over 5000 mg/kg by oral administration. Sodium salt of terephthalic acid was less toxic than the free acid; LD_{50} being 3600 mg/kg as terephthalic acid in sodium salt against 1430 mg/kg in the acid by intraperitoneal injection. LD_{50} of the salt by intravenous injection was more than 1000 mg/kg as the acid. The animals died of terephthalic acid intoxication, neither of peritonitis nor other diseases. The animals died within 48 hours after administration irrespective of the route. These results show that terephthalic acid and its sodium salt are practically non-toxic.

When animals were administered with terephthalic acid through intraperitoneal and subcutaneous routes, they died 2 to 24 hours after the administration, while they died 8 to 48

8) J.T. Litchfield, Jr. and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).
9) H.C. Scarborough and G.R. McKinney, *J. Med. Pharm. Chem.*, **5**, 175 (1962).
10) S. Reitman and S. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1957).
11) S.D. Kutob and G.L. Plaa, *Toxicol. Appl. Pharmacol.*, **4**, 354 (1962).
12) S.D. Kutob and G.L. Plaa, *J. Appl. Physiol.*, **17**, 123 (1962).
13) T.A. Scott and E.H. Melvin, *Anal. Chem.*, **25**, 1656 (1953).
14) A.G. Gornall, C. S. Bardamill, and M.M. David, *J. Biol. Chem.*, **177**, 751 (1949).
15) J.R. Spies, *J. Biol. Chem.*, **195**, 65 (1952).
16) H.J. Fister, "Manual of Standardized Procedures for Spectrophotometric Chemistry," 1950, Standard Scientific Supply Corp., New York.

Table I. Acute Toxicity of Terephthalic Acid in Mice

Route of administration	LD_{50} (mg/kg)	
	Terephthalic acid	Disodium salt
Oral	>5000	6300 (5000) [5600—7150]
Subcutaneous	—	8600 (6800) [7760—9550]
Intraperitoneal	1430 [1240—1650]	4600 (3600) [4260—4870]
Intravenous	—	1300 or more (1000 or more)

[ ] range at 95% of significance ( ) calculated dose as free acid

hours after oral administration. The animals, especially those surviving, showed no abnormal behavior except lethargy. No animals died after 3 to 7 days.

Effect of Terephthalic Acid feeding on Renal Function

The renal function was measured by the PSP retention test. As shown in Table II, the difference between treated and control was not significant, even though a slight depression of the dye excretion was observed in the administered group. It was found that the terephthalic acid feeding (815 mg/kg/day for 7 days) in this dose did not disturb the dye excretion from kidney.

Table II. Effect of Terephthalic Acid feeding on Renal Dye Excretion in Mice

Treatment	No. of animals	PSP content in plasma[a] (μg/ml)
Control	10	31.4±5.9
Terephthalic acid[b]	10	32.7±5.6[c]

a) mean value ± S.D.
b) Aniamls were fed with 0.5% terephthalic acid diet for 7 days (815 mg/kg/day).
c) not significant ($P > 0.05$)

Effect of Terephthalic Acid Injection on Renal Function

The effect of terephthalic acid injection on the dye excretion was examined. Terephthalic acid was injected intraperitoneally 25, 50, 100, or 200 mg/kg, and PSP was administered 45

Table III. Effect of Terephthalic Acid Injection on Renal Dye Excretion in Mice

Dose of terephthalic acid (mg/kg)	No. of animals	PSP cotent in blood plasma[a] (μg/ml)
0	10	31.4± 5.9
25	5	30.0± 6.3[b]
50	5	28.8± 5.9[b]
100	5	32.3± 4.1[b]
200	5	38.5± 8.4[b]
300	5	58.2±12.3[c]
500	5	65.6±12.6[c]
1000	5	85.6± 9.5[c]

a) mean value ± S.D
b) not significantly different from the control ($>P$ 0.05)
c) highly significant ($P < 0.001$) as compared to the control

min later. As shown in Table III, there was no significant difference among them and the control. However, after more than 300 mg/kg of the acid, increase in retention of plasma PSP was highly significant as compared with control.

Comparison of the Effects between Terephthalic Acid and Probenecid

In an effort to examine the effect of terephthalic acid on PSP excretion, the effect of Probenecid and the difference in their effects were studied.

As shown in Table IV, PSP was retained twice the control in plasma with 100 mg/kg of Probenecid, and with 300 mg/kg of terephthalic acid. This indicates that the acid has an effect on PSP retention in plasma, but that the effect was one-third of that of Probenecid.

Table IV. Effect of Probenecid Injection on Renal Dye Excretion in Mice

Dose of probenecid (mg/kg)	No. of animals	PSP content in blood plasma[a] (μg/ml)
0	10	31.4± 5.9
25	5	34.2± 6.3[b]
50	5	42.0±15.0[b]
100	5	56.4± 8.8[c]
200	5	70.1± 8.5[c]
300	5	75.4± 9.1[c]
500	5	89.2±14.0[c]
1000	2 died 3/5	110.5± 4.3[c]

a) mean value ± S.D.
b) not significantly different from the control ($P > 0.05$)
c) highly significant ($P < 0.001$) as compared to the control

Activity of Transaminases in Blood Plasma

GOT and GPT in Plasma were determined after terephthalic acid feeding (801 mg/kg/day for 7 days). The change in these enzyme activities was not recognized, as shown in Table V, and liver cells are thought to be not injured by terephthalic acid feeding.

Table V. Effect of Terephthalic Acid on Plasma Transaminase Activity in Mice

Treatment	No. of animals	Activity[a]	
		GOT	GPT
Control	15	59.4± 9.6	34.2± 8.9
Terephthalic acid fed[b]	15	59.3±14.5	34.5±11.1[c]

a) mean value ± S.D. Unit is Reitman-Frankel unit.
b) Animals were fed with 0.5% terephthalic acid diet for 7 days (801 mg/kg/day).
c) not significant $P > 0.05$)

Effect of Terephthalic Acid feeding on BSP Retention

The effect of terephthalic acid on the BSP excretion from liver was determined by the BSP retention test. Plasma content of BSP was assayed 15 min after the administration using 2 groups of 10 mice. The content in the administered group (852 mg/kg/day for 7 days) was found to be 6.3±1.8 (S.D.) mg/ml, and was significantly lower than the control (10.4±4.4, $P < 0.05$). Terephthalic acid did not retain the dye in plasma and rather accelerated its excretion.

Effect of Terephthalic Acid feeding on Barbiturate Sleeping-time

The barbiturate sleeping-time of mice was measured during terephthalic acid feeding. Mice were fed with 766 mg/kg/day of the acid for 7 days. The sleeping-time was rather

shortened by the acid feeding (Table VI). This suggests that the activation of barbiturate metabolism.

Table VI. Effect of Terephthalic Acid on Barbiturate Sleeping-time in Mice

Treatment	No. of animals	Sleeping-time[a] (min)
Control	35	10.2±4.0
Terephthalic acid fed[b]	20	6.6±2.4
Terephthalic acid injected[c]	10	5.9±2.2

a) mean value ± S.D.
b) Animals were fed with 0.5% terephthalic acid diet for 7 days (766 mg/kg/day).
c) Animals were injected 253 mg/kg of sodium salt of terephthalic acid (200 mg/kg as free acid) 24 hours before barbiturate administration.
d) highly significant ($P<0.01$) as compared to the control

Effect of Terephthalic Acid Injection on Barbiturate Sleeping-time

The sleeping-time was measured 24 hours after the subcutaneous injection of 253 mg/kg (200 mg/kg as the acid) of sodium terephthalate. The sleeping-time in the treated group was significantly shorter than control ($P<0.01$) (Table VI).

Effect of Terephthalic Acid feeding on Chemical Composition of Blood Plasma

Rats were used only in this experiment, and the differences in sugar, protein, free amino acid, and urea in blood plasma between the control and terephthalic acid fed group were investigated. The results are shown in Table VII and any significant difference was not recognized in the composition of above four items.

Table VII. Changes in Chemical Composition of Rat Blood Plasma during Terephthalic acid Feeding

Treatment	Content[a]			
	Sugar[b] (mg/dl)	Protein[c] (g/dl)	Free amino acid-nitrogen[d] (mg/dl)	Urea-nitrogen (mg/dl)
Control	156±7 (11)	5.93±0.23 (12)	22.4±2.8 (13)	23.2±1.8 (13)
Terephthalic acid fed[e]	158±3[f] (12)	5.89±0.21[f] (11)	22.4±2.0[f] (12)	22.5±2.1[f] (12)

a) mean value±S.D., No. of animals in parentheses
b) Values are calculated as glucose.
c) Values are calculated as albumin.
d) Values are calculated as L-alanine nitrogen.
e) Fed with 0.5% terephthalic acid diet for 7 days (310 mg/kg/day).
f) not significant ($P>0.05$)

Discussion

In previous papers on the metabolism of terephthalic acid, it has been shown that terephthalic acid is rapidly excreted intact in urine when it was injected intraperitoneally[6,17] and the contents of terephthalic acid in plasma and tissues were found to be at very low levels.[5,18] Studies on the growth and reproduction of mice fed with 0.5% terephthalic acid diet demon-

17) A. Hoshi and K. Kuretani, *Yakugaku Zasshi*, **85**, 905 (1965); A. Hoshi, J. Takagi, R. Yanai, and K. Kuretani, *Yakugaku Zasshi*, **86**, 963 (1966).
18) A. Hoshi, J. Takagi, and K. Kuretani, *Nippon Chikusan Gakkai-ho*, **37**, 115 (1966).

strated that the acid did not give any toxic effect on those animals.[7] In the present experiments, the acute toxicity of terephthalc acid and its sodium salt was examined by various administration routes. Toxicity of terephthalic acid is very low and its LD_{50} was more than 5000 mg/kg by oral administration. The sodium salt of terephthalic acid was less toxic than the acid itself, LD_{50} of the salt being 3600 mg/kg against 1430 mg/kg of the acid, by intraperitoneal injection. This would be explained by the acidity of terephthalic acid.

The effect of terephthalic acid on the renal function was studied by the PSP retention test, and there were no marked effect in the feeding experiment. However, when terephthalic acid was injected at a high dose of more than 300 mg/kg to the animals, PSP in blood plasma was retained significantly. This depression of the dye excretion from kidney was compared with the effect of Probenecid which is effective in enhancing the plasma content of penicillin,[19] *p*-aminosalicylic acid,[19] and PSP.[20] PSP was retained twice the control in plasma with 100 mg/kg of Probenecid, while it was similarly observed with about 300 mg/kg of terephthalic acid. LD_{50} of Probenecid is about 1000 mg/kg by intraperitoneal injection (Table IV) and that of terephthalic acid is 1430 mg/kg (Table I). Terephthalic acid has less effect on PSP excretion than Probenecid.

As the activities of GOT and GPT in blood plasma are increased by hepatocellular injury,[21] both enzymes were assayed. Activity of these enzymes remained unchanged after terephthalic acid administration, and the fact suggests that any liver injury was not caused by the acid. The BSP retention and the barbiturate sleeping-time were also determined during feeding and injection of terephthalic acid. If the liver functions for detoxication were decreased by the acid, the BSP retention and the barbiturate sleeping-time might be increased and elongated. Nevertheless, BSP retention was lowered and barbiturate sleeping-time was shortened by the acid feeding or injection. These results showed that the liver functions were not inhibited, but rather accelerated by the terephthalic acid administration.

As the effect of terephthalic acid over other physiological conditions of animals, the content of sugar, protein, free amino acid, and urea in boold plasma in the rat were examined. Blood sugar content is one of the most strictly regulated components of the homeostatic mechanisms, on which liver plays an essential rôle.[22] The plasma nitrogen compounds may be changeable by some kind of liver or kidney dysfunction.[22] These contents did not change under the terephthalic acid feedintg, and the fact suggested that both liver and kidney functions operated normally.

The present experiments showed that terephthalic acid and its sodium salt were not toxic at usual doses, and that the liver and renal functions were not toxically affected by the terephthalic acid administration.

Acknowledgement The authors are indebted to Dr. Zenzo Tamura of Tokyo University for his interest and encouragement in this work.

19) W.P. Boger, J.O. Beatty, F.W. Pitts, and H.F. Flippin, *Ann. Internal Med.*, **33**, 18 (1950).
20) K.H. Beyer, H.F. Russo, E.K. Tillson, A. K. Miller, W. F. Verwey, and S. R. Gass, *Am. J. Physiol.*, **166**, 625 (1951).
21) M. Chlinsky, G. L. Shmagranoff, and S. Sherry, *J. Lab. Clin. Med.*, **47**,108 (1956).
22) H.A. Harper, "Review of Physiological Chemistry," Lange Medical Publications, California, 1959.