Obstructive nephropathy induced with DL-potassium hydrogen tartrate in F344 rats

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Abstract: We experienced obstructive nephropathy in F344 rats treated with DL-potassium hydrogen tartrate (PHT) in a 13-week oral repeated dose toxicity study. Six-week-old male and female F344/DuCrj rats were fed a diet containing up to 2.0% PHT for 13 weeks. Microscopical findings including irregular dilation of the distal tubule lumen, foreign body giant cells, inflammatory cell infiltration, and regeneration of renal tubules were observed focally or multifocally in the renal cortex and/or medulla in the 0.5% and higher dosage groups of both sexes. The severity of these lesions increased in a dose-dependent manner. In the urinalysis, an increase in protein and white blood cells or the concentration of tartaric acid was detected in the 0.5% PHT and higher dosage groups of both sexes or males, respectively, though conventional blood biochemical analysis did not indicate failure of renal function. These results indicate that the PHT induces obstructive nephropathy in rats. There were no other treatment-related changes in other organs. (DOI: 10.1293/tox.2014-0058; J Toxicol Pathol 2015; 28: 89–97)

Key words: obstructive nephropathy, rat, kidney, DL-potassium hydrogen tartrate

Introduction

Obstructive nephropathy is known to be a chemically induced renal lesion caused by crystals formed in the renal tubules1. We found this nephropathy in F344 rats treated with up to 2.0% DL-potassium hydrogen tartrate (PHT) in a 13-week oral repeated dose toxicity study. PHT is registered as a food additive that is used as a flavor enhancer, a pH regulator and a leavening agent in Japan. It forms colorless crystals or a white crystalline powder, and the taste of its acid solution is cool and sour. The chemical is supplied as a mixture containing equivalent amount of D- and L- plus small amount of meso-tartaric acids. Although there are no toxicity studies in accordance with current test guidelines authorized by national/international organizations, previous studies indicated some evidence of renal toxicity caused by PHT 2–5. Therefore, to provide general toxicity information especially focusing on the renal toxicity of PHT, we conducted a 13-week oral repeated dose toxicity study of PHT in rats.

Materials and Methods

In this 13-week oral repeated dose toxicity study, six-week-old male and female F344/DuCrj rats (Charles River Laboratories Japan, Inc., Atsugi, Japan) randomly divided into 4 groups consisting of 10 males and 10 females were given 0% (control), 0.125%, 0.5%, and 2.0% PHT (Hangzhou Jin Tian Chemical Co., Ltd., Shanghai, PR China) in basal powdered diet for 13 weeks. PHT was supplied by the Japan Food Additive Association (Tokyo, Japan) and was mixed well with powdered basal diet, CRF-1 (Oriental Yeast Co., Tokyo, Japan), at each concentration. The doses of PHT in the present study were based on a dose-setting study previously performed. In the dose-setting study, 0% (control), 0.05%, 0.2%, 1.0% and 5.0% PHT were administered to 5 rats/group/sex for 4 weeks, and 5.0% PHT induced renal damage indicating severe obstructive nephropathy as well as significant increases in kidney weight and BUN in both sexes. Therefore, 2.0%, between 1.0% and 5.0%, was selected as the highest dose for the 13-week study. The test diet was available ad libitum, except for a one-night fasting period prior to scheduled sacrifice. The animals were checked daily for clinical signs and mortality, and body weights and food intake were measured every week during the study period. For urinalysis, fresh urine was collected from all animals at weeks 4 and 13 and was checked using test strips (Uropaper III Eiken, Eiken Chemical Co., LTD., Tochigi, Japan). Additionally, 20-hour urine samples from all males were collected using metabolic cages (Natsume Seisakusyo Co., Ltd., Tokyo Japan) at week 11 to quantitate
Table 1. Histopathological Findings in the Kidneys of F344 Rats Treated with DL-Potassium Hydrogen Tartrate (PHT) for 13 Weeks

| Findings                                                                 | Group          | Control | 0.125% PHT 10 | 0.5% PHT 10 | 2.0% PHT 10 (9) |
|-------------------------------------------------------------------------|----------------|---------|---------------|-------------|-----------------|
|                                                                     | No. of animals examined |         |               |             |                 |
| Males                                                                  |                |         |               |             |                 |
| Irregularly shaped lumen, distal tubules, cortex or medulla (focal/multifocal) | 0 0 1/0 4* | 0 0 1 0 | 0 0 1 4* |
| Foreign body giant cell with irregularly shaped phagocytic materials, focal, cortex or medulla | 0 0 0 4* | 0 0 0 4* | 0 0 0 4* |
| Foreign body giant cell with irregularly shaped phagocytic materials, inflammation and tubular regeneration in the surroundings (focal, cortex) | 10 10 10 9 | 10 10 10 9 | 10 10 10 9 |
| Tubular regeneration, proximal tubules, cortex and/or medulla (mild) | 3 2 2 0 | 3 2 2 0 | 3 2 2 0 |
| Mineralization, focal, cortex or papilla (mild) | 7 7 7 4 | 7 7 7 4 | 7 7 7 4 |
| Mononuclear cell infiltration, focal, interstitial, cortex or medulla (mild) | 0 0 0 1b | 0 0 0 1b | 0 0 0 1b |
| Severe dilatation of the pelvis with foreign body giant cell including irregularly shaped phagocytic materials, inflammation and tubular regeneration in the surroundings (diffuse, severe), unilateral | 2 4 5 5 | 2 4 5 5 | 2 4 5 5 |
| Foreign body giant cell with irregularly shaped phagocytic materials, inflammation and tubular regeneration in the surroundings (multifocal, cortex, contralateral) | 0 0 0 1b | 0 0 0 1b | 0 0 0 1b |
| Females                                                                 |                |         |               |             |                 |
| Irregularly shaped lumen, distal tubules, cortex or medulla (focal/multifocal) | 0 0 1/0 3/1 | 0 0 1/0 3/1 | 0 0 1/0 3/1 |
| Foreign body giant cell with irregularly shaped phagocytic materials, focal, cortex or medulla | 0 0 0 1 | 0 0 0 1 | 0 0 0 1 |
| Foreign body giant cell with irregularly shaped phagocytic materials, inflammation and tubular regeneration in the surroundings (focal/multifocal, cortex) | 2 4 5 5 | 2 4 5 5 | 2 4 5 5 |
| Tubular regeneration, proximal tubules, cortex and/or medulla (mild) | 8 9 10 6 | 8 9 10 6 | 8 9 10 6 |
| Mineralization, focal, cortex, medulla or papilla (mild) | 4 4 5 5 | 4 4 5 5 | 4 4 5 5 |
| Mononuclear cell infiltration, focal, interstitial, cortex or medulla (mild) | 0 0 0 1b | 0 0 0 1b | 0 0 0 1b |
| Nephroblastoma, unilateral | 0 0 0 1b | 0 0 0 1b | 0 0 0 1b |
| Irregularly shaped lumen, distal tubules, multifocal, cortex or medulla, bilateral | 0 0 0 1b | 0 0 0 1b | 0 0 0 1b |
| Foreign body giant cell with irregularly shaped phagocytic materials, inflammation and tubular regeneration in the surroundings (multifocal, cortex, bilateral) | 0 0 0 1b | 0 0 0 1b | 0 0 0 1b |

* Two animals (male No. 135 [male] and No. 240 [female]) were excluded due to heterogeneous type of lesion in the kidney. b) Excluded male (No. 135). b) Excluded female (No. 240). * Significantly different from controls at p<0.05 (Fisher’s exact probability test).

Results

No animals died or were euthanized when moribund during the treatment period. No treated-related clinical signs were observed. Mean body weight and food intake in all treated groups were comparable to those in the control group during the treatment period. However, the mean final body weight after fasting was significantly but slightly decreased (4.5%) in the male 2.0% group, compared with the control group (Table 3). At the necropsy, cystic enlargement in 1 male and a ball-shaped enlargement in 1 female were macroscopically observed in the unilateral kidney of the 2.0% groups. Other findings in the kidneys were not detected macroscopically. Detailed histopathological findings in the kidneys are summarized in Table 1. In the 2.0% groups, focal or multifocal irregular dilation of the distal tubule was found in the cortex or medulla in both sexes (Fig. 1A). In the same groups, foreign body giant cells with irregular phagocytic vacuoles (an asterisk in Fig. 1B) accumulated in the renal cortex or medulla. In more severe cases of the 2.0% groups, inflammatory cell infiltration and regeneration of renal tubules around the foreign body giant cells were found focally or multifocally and surrounded the damaged tubules (Fig. 1C). In 1 male of the 2.0% group exhibiting cystic enlargement in the unilateral kidney at necropsy, severe expansion in the renal pelvis compressed the remaining renal parenchyma, and inflammation, regeneration of renal tubules, and foreign body giant cells were...
evident and more severe than those in other animals. In the contralateral kidney of this male animal, multifocal lesions including inflammation with foreign body giant cells and regeneration of renal tubules were observed in the cortex, and several foreign body giant cells were also seen in the tip of the renal papilla. In the same animal affected with severe renal lesions, diffuse hyperplasia of the transitional epithelium was detected in the urinary bladder. In 1 female of the 2.0% group, a nephroblastoma was found as a ball-shaped kidney. Distal tubules with an irregular lumen, inflammation with foreign body giant cells, and regeneration of renal tubules were distinctly observed in both kidneys of this female animal compared with other animals in the same group.

In the 0.5% groups, irregularly dilated distal tubules and foreign body giant cells were focally seen in the cortex or medulla in 1 female and 1 male, respectively. The incidence and multiplicity of these findings in the 0.5% group were less than in the 2.0% groups. As for other lesions, mild regeneration of the proximal tubules, mineralization, and mononuclear cell infiltration were observed in all of the groups including controls, and, there were no significant differences in the incidences of these lesions between the controls and treated groups in both sexes.

From the urinalysis using test strips, a decreasing trend in pH for the male 2.0% group and an increasing trend in protein for the 0.5% and higher female dosage groups were observed at week 4 (Table 2). At week 13, an increase in protein and white blood cells in urine was detected for the 0.5% and higher PHT dosage groups of both sexes. In the quantitative analysis of tartaric acid in urine for males at week 11, the concentration of tartaric acid significantly increased in a dose-dependent manner for the 0.5% and higher PHT doses (Table 2). As for other changes related to the kidneys (Table 3), a significant increase in kidney weight (relative in males and both absolute and relative in females) was observed in the 2.0% groups of both sexes. In the serum biochemistry, renal damage markers such as BUN and CRN did not in-

**Fig. 1.** Histopathological findings in the kidneys of F344 rats treated with PHT for 13 weeks. (A) Irregularly shaped lumen of the distal tubule, (B) foreign body giant cell (asterisk) with irregularly shaped phagocytic material, (C) foreign body giant cell (arrowhead) with irregular shaped phagocytic material, inflammation, and tubular regeneration in the surrounding tissue. Bars = 50 μm.
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crease as result of PHT treatment. The levels of CRN at the 0.5% and higher doses in females and at 2.0% in males significantly decreased, but this might have been incidental.

There were no other treatment-related changes in hematological and serum biochemistry parameters or the results of histopathological examination in the organs except for the kidneys for the treated rats of both sexes (Tables 4–7).

Discussion

Internationally, risk assessment for tartaric acid and its potassium or sodium salt was performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (http://www.inchem.org/documents/jecfa/jecmono/v12je03.htm) in 1977. Some reports suggest that the kidney is the target of toxicity from tartaric acid and related chemicals in humans and animals. According to these reports, intravenous treatment with 0.2–0.3 g of tartaric acid caused renal disorder in rabbits and rats\(^2\).\(^3\). In humans, accidental intake of a large amount (30 g) of tartaric acid was lethal due to tubular nephropathy\(^4\). Moreover, 2.73 g/kg/day monosodium DL-tartrate was found to be accumulated in rat kidneys, resulting in increased kidney weight, renal toxicity, and death\(^5\). However, detailed histopathology of the kidneys was

| Table 2. Urinalysis Data and Tartaric Acid Concentration in the Urine at Weeks 4 and 13 in F344 Rats Treated with DL-Potassium Hydrogen Tartrate (PHT) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Week 4**      | **Control**     | **0.125% PHT**  | **0.5% PHT**    | **2.0% PHT**    |
| **Males**       | **(n=)**        | **(n=)**        | **(n=)**        | **(n=)**        |
| Protein         | −                | 0               | 0               | 0               |
| ±               | 0               | 0               | 2               | 0               |
| ++              | 5               | 6               | 4               | 9               |
| +++             | 1               | 0               | 0               | 0               |
| **pH**          | 5               | 0               | 0               | 0               |
|                | 6               | 2               | 2               | 8               |
|                | 7               | 4               | 6               | 0               |
|                | 8               | 3               | 1               | 1               |
| **Females**     | **(n=)**        | **(n=)**        | **(n=)**        | **(n=)**        |
| Protein         | −                | 0               | 0               | 0               |
| ±               | 6               | 6               | 0               | 0               |
| +               | 3               | 4               | 9               | 8               |
| ++              | 0               | 0               | 1               | 2               |
| +++             | 0               | 0               | 0               | 0               |
| **pH**          | 5               | 3               | 0               | 0               |
|                | 6               | 4               | 9               | 9               |
|                | 7               | 2               | 1               | 0               |
|                | 8               | 0               | 0               | 1               |
| **Week 13**     | **(n=)**        | **(n=)**        | **(n=)**        | **(n=)**        |
| **Males**       | −                | 7               | 6               | 8               |
| ±               | 1               | 1               | 3               | 2               |
| +               | 1               | 1               | 0               | 1               |
| ++              | 1               | 0               | 0               | 0               |
| **WBC**         | −                | 6               | 8               | 0               |
| ±               | 0               | 0               | 0               | 0               |
| +               | 2               | 4               | 2               | 7               |
| ++              | 0               | 0               | 0               | 0               |
| **Protein**     | −                | 0               | 0               | 0               |
| ±               | 0               | 0               | 0               | 0               |
| +               | 2               | 4               | 2               | 7               |
| ++              | 0               | 0               | 0               | 0               |
| **Glucose**     | −                | 10              | 10              | 10              |
| ±               | 10              | 0               | 0               | 0               |
| +               | 0               | 0               | 0               | 0               |
| ++              | 0               | 0               | 0               | 0               |
| **pH**          | 5               | 6               | 8               | 10              |
|                | 10              | 10              | 10              | 10              |
| **Tartaric acid (%)** | **0.061 ± 0.012** | **0.096 ± 0.024** | **0.189 ± 0.054** | **0.479 ± 0.219** |

\(^a\): One animal was excluded due to the difficulty of urine sampling. \(^b\): Mean ± SD. WBC, White blood cell. ** Significantly different from controls at p<0.01 (Dunnett’s test).
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PHT treatment clearly induced obstructive nephropathy in both the preliminary and present studies. The irregularly shaped lumen in the distal tubules might be due to obstruction from crystals related to PHT, which might form in the process of urine concentration at the distal tubules. The crystals formed in the distal tubules could damage the epithelium of the tubules, allowing the PHT to leak from the collapsed tubules and be incorporated by inflammatory reactions including in the giant cells. In the present study, the crystals of PHT or its metabolites could not be detected in the kidney sections; however, the crystals might have dissolved during tissue section preparation. The urinalysis data for WBCs and protein indicating damage of the renal tubules, acidification of urine, and an increase in kidney weight in the 2.0% groups were considered PHT-related changes. In the 0.5% groups, although treatment-related changes in body and kidney weights, serum BUN, and CRN were not observed, obstructive nephropathy was found in 1 female and 2 males, indicating that microscopic examination is highly sensitive for detection of obstructive nephropathy, and based on the microscopic findings, 0.5% PHT is deemed the toxic dose due to the significant renal changes. The mean concentration of renal damage markers such as BUN and CRN in the 0.5% groups were not affected by PHT treatment because only one animal of each sex showed

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nephropathy and the severity of the lesion was minimal. In the 2.0% groups, hydronephrosis and nephroblastoma were observed in 1 male and 1 female, respectively. Hydronephrosis is often observed in young rats as a hereditary disease, which usually affects the right kidney, and is also caused by stones blocking the renal pelvis, ureter, and bladder. No crystals of PHT were detected in the urinary tract, and hydronephrosis was observed in the unilateral (right kidney) of the corresponding animal in the present study, indicating that the lesion in the present study was spontaneous. Nephroblastoma is known to be a spontaneous tumor in young rats. No preneoplastic lesions were observed in the present study. Therefore, the 1 nephroblastoma in the female 2.0% group is considered to be spontaneous and not treatment-related. The obstructive nephropathy was more intense in the remaining parenchyma in the kidneys affected with hydronephrosis or nephroblastoma than in other animals in the same groups because of the decrease of its normal parenchyma. Diffuse hyperplasia in the urinary bladder of the animal exhibiting hydronephrosis might be related to physical stimulation by crystals from the kidneys. The concentration of tartaric acid significantly increased in the urine of males at doses greater than 0.5%. This result indicates that PHT might be excreted into urine, similar to other tartaric acids. It was reported that about 68% of L(+)-tartaric acid or 70% of sodium tartrate was excreted into urine when 400 mg/kg BW L(+)-tartaric acid or sodium tartrate was orally administered to rats (WHO Food Additive Series 12: http://www.inchem.org/documents/jecfa/jecmono/v12je03.htm). It was also reported that 7-day

### Table 4. Hematological Data of F344 Rats Treated with DL-Potassium Hydrogen Tartrate (PHT) for 13 Weeks

| Group          | Control | 0.125% PHT | 0.5% PHT | 2.0% PHT |
|----------------|---------|------------|----------|----------|
| **Males**      |         |            |          |          |
| No. of animals examined | 10      | 10         | 10       | 10       |
| RBC (×10^6/μL) | 879.2 ± 33.3a | 878.5 ± 44.9 | 882.3 ± 77.1 | 878.9 ± 45.5 |
| Hb (g/dL)      | 15.0 ± 0.5 | 15.0 ± 0.5 | 14.7 ± 1.4 | 14.8 ± 0.6 |
| Ht (%)         | 47.2 ± 1.6 | 47.1 ± 2.2 | 47.0 ± 4.0 | 47.0 ± 2.3 |
| MCV (fl)       | 53.6 ± 0.4 | 53.7 ± 0.4 | 53.3 ± 0.4 | 53.5 ± 0.5 |
| MCH (pg)       | 17.0 ± 0.3 | 17.1 ± 0.4 | 16.6 ± 0.3* | 16.9 ± 0.4 |
| MCHC (g/dL)    | 31.7 ± 0.5 | 31.8 ± 0.7 | 31.3 ± 0.7 | 31.5 ± 0.7 |
| Pt (×10^6/μL)  | 73.7 ± 8.8 | 69.9 ± 9.2 | 74.9 ± 7.6 | 79.0 ± 4.8 |
| WBC (×10^9/μL) | 50.0 ± 10.8 | 45.5 ± 6.6 | 51.0 ± 14.1 | 47.7 ± 9.6 |
| **Differential leukocyte counts** |         |            |          |          |
| No. of animals examined | 9b       | 10         | 10       | 9b       |
| Band form neutrophils (%) | 1.4 ± 0.5 | 1.3 ± 0.7 | 1.3 ± 0.7 | 0.9 ± 0.6 |
| Segmented neutrophils (%) | 27.0 ± 5.3 | 28.1 ± 5.1 | 30.4 ± 7.9 | 25.4 ± 6.4 |
| Eosinophils (%) | 1.0 ± 0.7 | 0.9 ± 0.7 | 1.4 ± 0.7 | 1.1 ± 0.9 |
| Basophils (%)   | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Lymphocytes (%) | 70.1 ± 5.4 | 69.0 ± 5.9 | 66.6 ± 8.2 | 72.1 ± 7.3 |
| Monocytes (%)   | 0.5 ± 0.4 | 0.6 ± 0.4 | 0.4 ± 0.3 | 0.5 ± 0.5 |
| Reticulocytes (/100 cells) | 0.06 ± 0.17 | 0.06 ± 0.17 | 0.10 ± 0.21 | 0.05 ± 0.17 |
| **Females**    |         |            |          |          |
| No. of animals examined | 9b       | 10         | 10       | 9b       |
| RBC (×10^6/μL) | 849.8 ± 39.0 | 856.2 ± 44.0 | 846.5 ± 33.3 | 842.7 ± 31.0 |
| Hb (g/dL)      | 15.3 ± 0.6 | 15.3 ± 0.7 | 15.1 ± 0.7 | 15.1 ± 0.7 |
| Ht (%)         | 47.8 ± 2.2 | 47.9 ± 2.6 | 47.3 ± 1.9 | 46.9 ± 1.8 |
| MCV (fl)       | 56.1 ± 0.3 | 56.0 ± 0.4 | 55.9 ± 0.3 | 55.7 ± 0.4* |
| MCH (pg)       | 18.0 ± 0.3 | 17.8 ± 0.5 | 17.8 ± 0.3 | 17.9 ± 0.3 |
| MCHC (g/dL)    | 32.2 ± 0.4 | 31.8 ± 0.9 | 31.9 ± 0.6 | 32.2 ± 0.5 |
| Pt (×10^6/μL)  | 69.3 ± 13.4 | 71.0 ± 13.8 | 77.1 ± 3.9 | 80.7 ± 4.4* |
| WBC (×10^9/μL) | 30.4 ± 7.6 | 28.6 ± 5.4 | 29.4 ± 6.2 | 28.0 ± 8.3 |
| **Differential leukocyte counts** |         |            |          |          |
| No. of animals examined | 8b       | 9b         | 10       | 9b       |
| Band form neutrophils (%) | 0.8 ± 0.4 | 1.2 ± 0.6 | 1.6 ± 1.0* | 1.5 ± 0.6* |
| Segmented neutrophils (%) | 27.6 ± 5.4 | 28.6 ± 3.8 | 26.4 ± 5.1 | 26.5 ± 4.5 |
| Eosinophils (%) | 1.1 ± 1.1 | 1.0 ± 0.7 | 1.4 ± 0.9 | 1.5 ± 0.9 |
| Basophils (%)   | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Lymphocytes (%) | 69.4 ± 5.5 | 68.5 ± 4.0 | 70.2 ± 4.8 | 69.8 ± 4.2 |
| Monocytes (%)   | 1.1 ± 0.3 | 0.7 ± 0.6 | 0.5 ± 0.4 | 0.7 ± 0.5* |
| Reticulocytes (/100 cells) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.05 ± 0.16 | 0.06 ± 0.17 |

*a: Mean ± SD. b: One or two animals were excluded due to an insufficient blood sample. RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Pt, platelet; WBC, white blood cell. Significant difference from the controls (Dunnett’s test or Steel’s test) are indicated by asterisks. *p<0.05.
treatment of rats with high-dose (14C)-labeled DL-sodium tartrate (2.73 g/kg BW) resulted in an increase in relative weight and accumulation of the compound in the kidneys (WHO Food Additive Series 12: http://www.inchem.org/documents/jecfa/jecmono/v12je03.htm). This evidence supports our speculation that tartaric acid and/or its metabolites form crystals that accumulate in the kidney in the process of urine concentration and that the crystals damage the tubules in the process of excretion, which might cause obstructive nephropathy.

Other findings from the kidneys including regeneration of the proximal tubules, mineralization, and mononuclear cell infiltration in the interstitium or from the other organs observed in all of the groups were not dose dependent, indicating that they were spontaneous or incidental lesions.

The present study is the first toxicity study conducted in accordance with current test guidelines from national/international organizations. According to the data of the present study, the no-observed-adverse-effect level (NOAEL) is judged to be 0.125% in both sexes (males, 0.075 g/kg body weight/day; females, 0.082 g/kg body weight/day). According to an old report, a large amount of tartaric acid (30 g) is lethal to humans due to tubular nephropathy. The present data indicate that DL-potassium hydrogen tartrate has inducible renal toxicity in humans exposed to a high dose. Therefore, further toxicological information for other tartrates is necessary.

Table 5. Serum Biochemical Data of F344 Rats Treated with DL-Potassium Hydrogen Tartrate (PHT) for 13 Weeks

| Group       | Control | 0.125% PHT | 0.5% PHT | 2.0% PHT |
|-------------|---------|------------|----------|----------|
| Males       |         |            |          |          |
| No. of animals examined | 10      | 10         | 10       | 10       |
| TP (g/dL)   | 6.40 ± 0.18a | 6.44 ± 0.13 | 6.45 ± 0.12 | 6.48 ± 0.18 |
| A/G         | 2.12 ± 0.12  | 2.11 ± 0.14 | 2.07 ± 0.11 | 2.02 ± 0.08  |
| Alb (g/dL)  | 4.34 ± 0.08  | 4.37 ± 0.13 | 4.34 ± 0.05 | 4.34 ± 0.14  |
| Total Bil (mg/dL) | 0.034 ± 0.008 | 0.032 ± 0.010 | 0.036 ± 0.008 | 0.034 ± 0.007 |
| Glucose (mg/dL) | 156.6 ± 15.1 | 159.0 ± 14.7 | 154.4 ± 10.4 | 163.8 ± 23.0 |
| TG (mg/dL)  | 109.0 ± 23.0 | 101.8 ± 34.5 | 105.1 ± 17.3 | 99.5 ± 34.1  |
| TC (mg/dL)  | 65.4 ± 4.1    | 64.7 ± 4.3   | 65.5 ± 4.3  | 69.0 ± 6.4   |
| Na (mEQ/L)  | 142.5 ± 0.7   | 142.6 ± 0.8  | 141.8 ± 1.4 | 141.4 ± 1.6  |
| Cl (mEQ/L)  | 103.1 ± 0.9   | 103.7 ± 2.1  | 103.4 ± 1.2 | 102.9 ± 1.0  |
| K (mEQ/L)   | 4.30 ± 0.24   | 4.29 ± 0.11  | 4.29 ± 0.15 | 4.24 ± 0.13  |
| Ca (mg/dL)  | 10.07 ± 0.13  | 10.20 ± 0.18 | 10.07 ± 0.13 | 10.17 ± 0.23  |
| IP (mg/dL)  | 5.15 ± 0.40   | 4.99 ± 0.56  | 4.93 ± 0.31 | 4.87 ± 0.66  |
| AST (IU/L)  | 79.6 ± 9.3    | 76.6 ± 6.9   | 85.8 ± 15.3 | 74.1 ± 13.2  |
| ALT (IU/L)  | 52.7 ± 7.4    | 49.5 ± 3.9   | 53.8 ± 8.4  | 48.6 ± 6.1   |
| ALP (IU/L)  | 413.0 ± 33.9  | 438.7 ± 17.2 | 448.2 ± 26.2 | 437.0 ± 24.7 |
| γ-GTP (IU/L) | <3        | <3          | <3        | <3        |
| Females     |         |            |          |          |
| No. of animals examined | 10      | 10         | 10       | 10       |
| TP (g/dL)   | 6.27 ± 0.19  | 6.21 ± 0.22 | 6.13 ± 0.23 | 6.11 ± 0.12 |
| A/G         | 2.45 ± 0.20  | 2.45 ± 0.14 | 2.53 ± 0.14 | 2.25 ± 0.14a|
| Alb (g/dL)  | 4.45 ± 0.12  | 4.41 ± 0.15 | 4.39 ± 0.16 | 4.23 ± 0.13a|
| Total Bil (mg/dL) | 0.058 ± 0.015 | 0.053 ± 0.012 | 0.048 ± 0.010 | 0.045 ± 0.012 |
| Glucose (mg/dL) | 110.9 ± 15.0 | 115.8 ± 14.4 | 134.1 ± 16.8a | 137.2 ± 17.6a |
| TG (mg/dL)  | 24.5 ± 7.6   | 27.1 ± 11.7 | 20.6 ± 4.8  | 26.7 ± 10.0  |
| TC (mg/dL)  | 88.6 ± 5.9   | 85.4 ± 7.2  | 87.0 ± 7.1  | 88.9 ± 7.5   |
| Na (mEQ/L)  | 142.1 ± 0.9  | 141.2 ± 1.4 | 141.1 ± 1.8 | 141.5 ± 1.4  |
| Cl (mEQ/L)  | 105.5 ± 1.3  | 104.2 ± 1.5 | 103.6 ± 1.2a | 103.5 ± 1.4a |
| K (mEQ/L)   | 4.16 ± 0.25  | 4.09 ± 0.31 | 4.08 ± 0.19 | 4.28 ± 0.35  |
| Ca (mg/dL)  | 9.53 ± 0.13  | 9.49 ± 0.26 | 9.34 ± 0.28 | 9.42 ± 0.22  |
| IP (mg/dL)  | 4.92 ± 0.44  | 5.01 ± 0.52 | 4.90 ± 0.55 | 4.99 ± 0.42  |
| AST (IU/L)  | 75.9 ± 3.7   | 71.4 ± 6.7  | 71.9 ± 11.9 | 72.5 ± 8.9   |
| ALT (IU/L)  | 37.3 ± 3.8   | 35.1 ± 5.3  | 33.4 ± 4.9  | 34.0 ± 4.2   |
| ALP (IU/L)  | 278.3 ± 24.3 | 271.9 ± 30.9 | 273.8 ± 33.6 | 284.8 ± 41.8 |
| γ-GTP (IU/L) | <3        | <3          | <3        | <3        |

* Mean ± SD. TP, total protein; A/G, albumin: globulin ratio; Alb, albumin; Bil, bilirubin; TG, triglyceride; TC, total cholesterol; Na, sodium; Cl, chloride; K, potassium; Ca, calcium; IP, inorganic phosphate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, gamma glutamyl transpeptidase. Significant differences from the controls (Dunnett's test or Steel's test) are indicated by asterisks. *p<0.05; **p<0.01.
Table 6. Relative Organ Weights of F344 Rats Treated with DL-Potassium Hydrogen Tartrate (PHT) for 13 Weeks

| Group            | Control | 0.125% PHT | 0.5% PHT | 2.0% PHT |
|------------------|---------|------------|----------|----------|
| No. of animals examined | 10     | 10         | 10       | 10       |
| Males            |         |            |          |          |
| Brain (g/100 g BW) | 0.61 ± 0.02a | 0.62 ± 0.03 | 0.60 ± 0.02 | 0.62 ± 0.03 |
| Thymus (g/100 g BW) | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.06 ± 0.01 |
| Heart (g/100 g BW) | 0.28 ± 0.01 | 0.27 ± 0.01 | 0.27 ± 0.01 | 0.28 ± 0.01 |
| Lungs (g/100 g BW) | 0.33 ± 0.03 | 0.33 ± 0.02 | 0.32 ± 0.03 | 0.33 ± 0.04 |
| Spleen (g/100 g BW) | 0.20 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.01 | 0.21 ± 0.01 |
| Liver (g/100 g BW) | 2.40 ± 0.04 | 2.43 ± 0.11 | 2.50 ± 0.04** | 2.54 ± 0.09** |
| Adrenals (mg/100 g BW) | 13.0 ± 0.8 | 14.0 ± 2.3 | 11.5 ± 1.1† | 12.3 ± 1.4 |
| Testes (g/100 g BW) | 0.96 ± 0.04 | 0.97 ± 0.05 | 0.93 ± 0.04 | 0.96 ± 0.07 |
| Females          |         |            |          |          |
| Brain (g/100 g BW) | 1.05 ± 0.03 | 1.04 ± 0.05 | 1.05 ± 0.03 | 1.04 ± 0.06 |
| Thymus (g/100 g BW) | 0.09 ± 0.01 | 0.09 ± 0.01 | 0.08 ± 0.03 | 0.09 ± 0.01 |
| Heart (g/100 g BW) | 0.32 ± 0.01 | 0.32 ± 0.01 | 0.31 ± 0.01 | 0.31 ± 0.02 |
| Lungs (g/100 g BW) | 0.44 ± 0.03 | 0.44 ± 0.04 | 0.44 ± 0.04 | 0.44 ± 0.03 |
| Spleen (g/100 g BW) | 0.23 ± 0.01 | 0.23 ± 0.01 | 0.23 ± 0.01 | 0.24 ± 0.02 |
| Liver (g/100 g BW) | 2.20 ± 0.08 | 2.19 ± 0.14 | 2.26 ± 0.09 | 2.29 ± 0.14 |
| Adrenals (mg/100 g BW) | 25.5 ± 2.1 | 21.2 ± 4.2** | 23.8 ± 2.3 | 23.9 ± 2.6 |
| Ovaries (mg/100 g BW) | 26.2 ± 3.8 | 26.4 ± 2.4 | 24.3 ± 4.0 | 22.3 ± 3.9 |

Mean ± SD. BW, body weight. Significant difference from the controls (Dunnett's test or Steel's test) are indicated by asterisks. *p<0.05, **p<0.01.

Table 7. Other Histopathological Findings in F344 Rats Treated with DL-Potassium Hydrogen Tartrate (PHT) for 13 Weeks

| Findings                        | Group                        | Control | 2.0% PHT |
|---------------------------------|------------------------------|---------|----------|
|                                 | No. of animals examined      | 10      | 10       |
| Males                           |                              |         |          |
| Liver                           | Mononuclear cell infiltration, focal, minimal | 9       | 7        |
| Pancreas                        | Atrophy, exocrine glands, focal, mild | 3       | 2        |
|                                | Mononuclear cell infiltration, focal, minimal | 0       | 1        |
| Heart                           | Mononuclear cell infiltration, focal, minimal | 10      | 8        |
| Bladder                         | Hyperplasia, diffuse, transitional epithelium, mild | 0       | 1        |
| Testes                          | Degeneration, focal, seminiferous tubules, mild | 0       | 1        |
| Eyes                            | Atrophy, focal, retina       | 1       | 0        |
| Harderian gland                 | Mononuclear cell infiltration, focal, minimal | 1       | 0        |
| Females                         |                              |         |          |
| Liver                           | Mononuclear cell infiltration, focal, minimal | 10      | 9        |
| Pancreas                        | Mononuclear cell infiltration, focal, minimal | 3       | 2        |
| Heart                           | Mononuclear cell infiltration, focal, minimal | 9       | 7        |
| Eyes                            | Atrophy, focal, retina       | 1       | 0        |
| Harderian gland                 | Mononuclear cell infiltration, focal, minimal | 2       | 3        |
| Ovaries                         | Granuloma, focal            | 1       | 1        |
| Bone marrow                     | Granuloma, multifocal       | 2       | 0        |

Declaration of Conflicting Interests: We have no conflicts of interest.

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