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Chronic trimethyltin chloride exposure and the development of kidney stones in rats

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Abstract

We recently reported that occupational exposure to trimethyltin (TMT) is a risk factor for developing kidney stones. To further examine the association between TMT exposure and the formation of kidney stones, we conducted a 180-day animal study and exposed the randomly grouped Sprague–Dawley (SD) rats to TMT in the drinking water at doses of 0, 8.2, 32.8 and 131.3 $\mu\text{g kg}^{-1} \text{ day}^{-1}$. Transient behavioral changes were observed in the high-dose group during the first 2 weeks of exposure. TMT exposure led to a significant dose-dependent inhibition of renal H^+/K^+ -ATPase and an increase in urinary pH. In comparison to no kidney stones being identified in the control and the lowest dose group, 1 rat in the 32.8 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ dose group and 3 out of 9 rats in the 131.3 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ dose group were found to have stones in the kidney/urinary tract. Pathological analysis showed that more wide spread calcium disposition was observed in kidneys of rats with TMT exposure compared with the rats in the control group. However, X-ray diffraction (XRD) analysis found that the kidney stones were mainly composed of struvite with the formula: $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$, while calcium-containing components were also detected. Together, this study further demonstrates through animal studies that chronic exposure to a relatively low level of TMT induces nephrotoxicity and increases the risk for developing kidney stones.

Keywords

H^+/K^+ -ATPase; kidney stone; nephrotoxicity; reproductive/developmental toxicity; trimethyltin chloride

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Conflict of interest

The Authors did not report any conflict of interest.

Introduction

Methyltin mercaptides are widely used as heat and light stabilizers in the manufacturing of polyvinyl chloride (PVC) products (Hoch, 2001). They counteract the tendency of PVC to decompose upon heating or on prolonged exposure to light as a result of a loss of hydrochloric acid from the PVC polymer. Methyltin mercaptides are made from monomethyltin and dimethyltin (MMT and DMT) that are synthesized by a direct chemical reaction. During this process, trimethyltin (TMT) is also produced as a by-product. TMT can also be converted from DMT in vivo (Furuhashi *et al.*, 2008; Tang *et al.*, 2013a).

The impact of methyltin compounds on human health is primarily focused on the neurotoxic activity of TMT (McCollister and Schober, 1975; Kimbrough, 1976; Chang, 1990; Aschner and Aschner, 1992; Appel, 2004; Tang *et al.*, 2008; Tang *et al.*, 2010). Methyltins can be produced in the environment by bacterial actions, but human exposure is usually an occupational risk and most commonly occurs in workplaces where methyltins are manufactured or being used. For example, with the increasing use of methyltin as stabilizers in PVC production in developing countries, such as in China, multiple acute poisoning accidents in the workplace have been reported in recent years (Tang *et al.*, 2010). Other than the acute accidental exposure, there has been increasing concern for workers and the general public that may be chronically exposed to methyltin compounds. Methyltin compounds are the dominant heat stabilizers used in the PVC products manufactured in the US (Dobson *et al.*, 2005). As a result, water soluble methyltin compounds can be leached from PVC products, leading to the contamination of foodstuffs, beverages, drinking water, municipal water and sewage sludge (Sadiki and Williams, 1999; Hoch, 2001). Moreover, we recently showed that TMT has a 15-day half-life in rats (Tang *et al.*, 2013a), suggesting that methyltin compounds could accumulate in the human body after chronic exposure.

In a recent epidemiological investigation, we observed a threefold increase in the prevalence of kidney stones in workers chronically exposed to TMT in comparison with control workers (Tang *et al.*, 2013b). There was a positive association between the prevalence of kidney stones and the length of employment in the plants using chlorinated methyltinmercaptides as the heat stabilizer for PVC production. Previously we also reported that TMT can inhibit the activity of H^+/K^+ -ATPase in renal intercalated cells, and subsequently affect the regulation of urinary pH and minerals (Tang *et al.*, 2010), known risk factors for kidney stone formation (Wagner and Mohebbi, 2010). We thus hypothesize that chronic exposures to TMT inhibits H^+/K^+ -ATPase, which could lead to the alteration of urinary pH and electrolytes and subsequently an increase in the risk of developing kidney stones.

In the current study, we performed an animal study and exposed rats to various concentrations of TMT in the drinking water for 180 days. The aim of the present study was to test this hypothesis and examine the association between chronic TMT exposure and the risk of developing kidney stones.

Materials and Methods

Chemicals

TMT was purchased from Fisher Scientific (Pittsburgh, PA, USA) and dissolved in 0.9% saline, and fresh solutions were prepared daily and used for the study. Sodium acetate and Sodium tetraborate were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA), and used to prepare buffers for urinary and blood TMT analysis.

Animals

Our previous analysis found that the toxic effects induced by TMT treatment were comparable between male and female rats (Tang et al., 2010). Thus, only female Sprague–Dawley (SD, 70–90g) rats were used and purchased from Guangdong Medical Laboratory Animal Center, China (Certification number: 0055283). After an acclimation period of 7days, the animals were transferred and housed, three or four per cage, in stainless-steel wire cages without bedding in an approved animal facility with controlled temperature (18–26°C), humidity (50 ± 20%) and a 12-h light/12-h dark cycle. All animals were provided a standard diet and acidified water ad libitum. The experimental protocol was approved by the Animal Care and Use Committee of Guangdong Poison Control Center (GDPCC) in China.

TMT Treatment in Rats

Forty-four female SD rats (70–90g) were randomly assigned to four groups, including one control group and three treatment groups. The dose selection was based on our previous study that showed an acute LD₅₀ of 14.7 mg kg⁻¹ and a cumulative coefficient (Kcum) of 1.67, which characterized the cumulative properties of TMT (Tang *et al.*, 2013b). Rats were exposed to TMT through drinking water for a period of 180 days at doses of 0, 8.20, 32.8 and 131.3 µg per kg body weight per day (µg kg⁻¹ day⁻¹). The designated cumulative dose of TMT in each treatment group is approximately 0, 0.1, 0.4 and 1.7-fold of the acute LD₅₀ dose, respectively. The TMT was dissolved and suspended in distilled water. Our pilot analysis indicated that the aqueous TMT was stable for at least 3 months at 4°C. In this study, the TMT stock solution was freshly prepared each month and stored at 4°C. The drinking water containing TMT was prepared from the stock solution and refreshed every other day and the consumption of water was recorded. The body weight of each animal was measured every other week.

Urine Collection and Renal Function Analysis

At 90days and the end of the experiment (180 days) after TMT exposure, the animals were placed in metabolic cages to analyze renal function. The animals were subjected to a 24-h urine collection to determine urine volume, specific gravity and pH. Urinary creatinine concentration was analyzed by a 7080 Biochemical Automatic Analyzer (Hitachi Co., Tokyo, Japan).

Measurement of Blood and Urinary TMT by Gas Chromatography–Mass Spectroscopy (GC–MS)

At 180-days after TMT exposure, blood was collected in heparin-containing tubes from the retro-orbital venous plexus of animals. The level of TMT in urine and blood was analyzed by GC-MS using an Agilent 6890N gas chromatograph connected to an Agilent 5973 MSD mass spectrometer with a chemical ionization (CI) source (Santa Clara, CA, USA). The urine and blood samples were prepared for analysis as described earlier (Tang *et al.*, 2013a). Briefly, 2 or 5 ml of sodium acetate buffer (pH = 3.9) was added to 0.5 ml of whole blood or blood cells and plasma isolated from approximately 2 ml of whole blood or 5 ml of urine. Next, 7 ml of acetonitrile was then added into the mix of blood and urine. All samples were fully mixed for 5 min, and centrifuged at 4,500 g for 5 min, and the supernatant was collected and transferred into a sterilized 15-ml tube. Then 200 μ l of 1% sodium tetraborate buffer and 1 ml of n-hexane were added to supernatants. After mixing, the organic phase (upper layer) of whole blood and urine samples were then collected and 1 μ l of the hexane extract was injected in the splitless mode on a non-polar HP-5 ms GC column (60.0 m, 0.32 mm and 1.00 μ m) (Agilent Technologies Co. Ltd.). Helium was used as the carrier gas and maintained at a constant flow rate of 2.0 ml min⁻¹. The inlet temperature was 250°C. The GC oven temperature program applied was as follows: from 50°C (1 min) to 200°C at 20°C per min. The MS interface temperature was maintained at 200°C. The characteristic ion fragments of TMT have been previously identified in the pilot study, and the ion fragments of TMT in the scan mode were selected and the level of TMT in the test samples was calculated from a standard curve. The minimum detection limit for this method was 0.1 μ g l⁻¹, and the relative recovery of TMT from standards were in the range of 84.3–104.0% and extraction recovery of TMT from test samples were in the range of 91.5–101.9%.

Organ/Tissue Collection and Kidney Gross Anatomic and Histology Analysis

At the end of experiments on the 180th day, all rats were euthanized individually in a CO₂ chamber. All tissues including kidneys were quickly removed and weighed and relative organ weights and the coefficient of each organ/tissue was calculated using the formula: weight of each organ or tissue/weight of each rat $\times$ 100%.

Kidneys and urinary tract were observed macroscopically for stone formation. Kidneys were then quickly fixed in 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C overnight. Post-fixed kidneys were embedded in paraffin and sectioned to include all major structural landmarks of the kidney in each section. The paraffin-embedded kidney sections were deparaffinized and rehydrated and then stained with hematoxylin and eosin. An experienced pathologist who was blinded to the treatments conducted the pathological assessment by evaluating the kidney sections.

Colorimetric Determination of H⁺/K⁺-ATPase Activities

H⁺/K⁺-ATPase activity kits were purchased from Jiancheng Bio. (Nanjing, China) (Ahmed *et al.*, 2013). Rat kidney specimens (2–6 g) were weighted and placed in ice-cold saline buffer solution (1: 9, weight/volume ratio). Kidney tissues were minced with scissors and homogenized in a manual homogenizer. The resultant suspension was centrifuged for 10 min at 1,350 g. The supernatant was then collected and protein concentration was measure

using the Bradford protein assay. The H^+/K^+ -ATPase activities of kidney supernatants were determined by colorimetry according to the manufacturer's protocol. Enzyme activity was expressed in units defined as μmol inorganic phosphate per μg protein per hour ($\mu\text{mol h}^{-1} \text{mg}^{-1}$ protein).

Mineralogical Analysis of Kidney Stones by X-ray Diffraction (XRD) Analysis

The kidney stones were removed from rats, washed with distilled water to remove possible contamination and then dried at 100°C for about 3–4 h. The mineralogical analysis of kidney stones was conducted by X-ray diffraction (XRD) using a D/MAX-3A X-Ray Powder Diffractometer (Rigaku XRD) using Cu K-alpha radiation.

Statistical Analysis

Results are shown as mean $\pm$ standard deviation (SD). Comparisons between the group means were made by the one-way analysis of variance. The Mann–Kendall trend test was also used in the analysis. Statistically significance was set at $P < 0.05$.

Results

Overall assessment of TMT Exposure

Figure 1 shows the consumption of drinking water containing TMT by rats in all groups during the entire study period. After 35 days of TMT exposure, the consumption of drinking water by rats in the $131.3 \mu\text{g kg}^{-1} \text{day}^{-1}$ group was higher in comparison with rats in other groups. To ensure the correct dose of exposure in each group, we thus adjusted the concentration of TMT in the drinking water in the $131.3 \mu\text{g kg}^{-1} \text{day}^{-1}$ group based on the water consumption volume and body weight of rats. As shown in Table 1, no TMT was detected in urine or in the blood of rats in the control group. The TMT concentration in the urine and blood of rats in each treatment group increased in proportion to the doses of TMT given through drinking water (Table 1). Moreover, the data indicate that TMT has a higher affinity for red blood cells, with about 98% of the TMT in whole blood distributing to the red blood cells and only 2% remaining in plasma (Table 1). Table 2 shows that the daily intake of TMT over 180 days in each group was equivalent to a cumulative dose corresponding to approximately 0.1, 0.4 and 1.8-fold of the acute LD_{50} dose (mg kg^{-1}).

General Conditions of Rats Post TMT Exposure

TMT treatment of rats affects the consumption of drinking water, with the high-dose group having increased water consumption over the entire study period (Fig. 1). However, no changes in body weight were observed in the high-dose group compared with other groups over the 180-day study period (Fig. 2). In the $131.3 \mu\text{g kg}^{-1} \text{day}^{-1}$ high-dose group, several rats showed changes in behaviors, including increased irritability, vocalization, jumping and biting at the beginning of the treatment. These symptoms lasted approximately 2 weeks and recovered thereafter. The first death was recorded on the 77th day in the highest dose group, and the second death was found on day 112 in the same group. Gross anatomical analysis of both rats showed hemorrhages in the digestive tract and in the liver and kidney stones in one of the rats. Importantly, the two rats were among the rats that showed abnormal behaviors after TMT treatment; however, it is unclear whether these symptoms were related to

mortality. No abnormal behavioral responses or deaths were observed in all other groups during the entire study period. At the end of the 180-day exposure period, no significant changes in relative organ weights were observed with the exception of a significant decrease in uterus weight in all three treatment groups (Table 3).

Impacts of TMT Exposure on Renal Function

Figure 3 illustrates that TMT exposure results in a dose-dependent decrease in renal H^+/K^+ -ATPase activity (P-trend value < 0.05). Table 4 provides basic data of renal function analysis. TMT exposure led to a significant decrease in the concentration of urine creatinine of rats in the 32.8 and 131.3 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ dose groups, which was probably associated with the increase in urine volume observed in these two groups (Table 4). TMT exposure also significantly increased the urinary pH of rats in the same two high-dose groups when compared with the rats in the control group. No differences were observed for urine-specific gravity between the groups.

Kidney/urinary Tract Stones and Renal Pathology

One rat in the 32.8 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ dose group developed kidney stones. In the 131.3 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ dose group, stones were found in 3 out of 9 rats (Table 5). Several rats developed stones both in the kidney and urinary tract, and the largest stone (diameter up to 7mm) was found in urinary tract. Other significant pathological changes were observed in the kidney of rats treated with TMT compared with rats in the control group (Table 5 and Fig. 4). Overall, no abnormalities were found in kidneys from the control rats, with the exception of a few showing calcium deposits (Fig. 4A–C). In comparison, kidneys in rats with TMT exposure were found to have several pathological changes, including calcium deposits (Fig. 4D), renal tubular dilation (Fig. 4E), epithelial cell proliferation (Fig. 4F), protein deposition in the renal tubule (Fig. 4G) and inflammatory infiltration (Fig. 4H). Anatomical analysis of the urinary system found that the stones developed in the kidneys and in urinary tracts (Fig. 4I & J). XRD analysis showed that these kidney stones were mainly composed of phosphate minerals ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) although calcium was also detected.

Discussion

Previously, we showed that TMT can directly inhibit H^+/K^+ -ATPase in renal intercalated cells (Tang et al., 2010). The inhibition of renal H^+/K^+ -ATPase can reduce urine K^+ reabsorption, leading to hypokalemia. Over 68% of the patients from the TMT poisoning accidents developed hypokalemia before other symptoms of the central nervous systems were observed (Tang et al., 2008). Another direct consequence of the renal H^+/K^+ -ATPase inhibition is a reduced secretion of H^+ from urine by the kidney, which leads to renal tubular acidity and an increase of urinary pH, a known risk factor for kidney stone formation (Wagner and Mohebbi, 2010). In a recent cross-sectional epidemiologic study, we reported for the first time that an increased prevalence of kidney stones was observed in workers with TMT exposure (Tang *et al.*, 2013b), suggesting that chronic TMT exposure is a risk factor for the kidney stone formation. In the current chronic rat study, we confirmed our hypothesis and showed that chronic exposure to relatively low levels of TMT could lead to a dose-dependent increase in kidney stone formation in rats.

Neurobehavioral symptoms are the main concern for cases of acute TMT poisoning (Aldridge *et al.*, 1981; Bouldin *et al.*, 1981; Robertson *et al.*, 1987). Similarly, animal studies of TMT toxicity have also focused on acute exposure and neurotoxicity, with only a few exceptions (Dobson *et al.*, 2005; Trabucco *et al.*, 2009). With the improved manufacturing operations worldwide, acute and high level exposure to methyltin compounds in the workplace occurs much less frequently in the past. However, with the wide spread use of PVC products containing methyltin stabilizers in the workplace and in daily life, there is an increasing chance for workers and the general public to be chronically exposed to low levels of methyltin compounds. According to a report from the vinyl institute (<http://www.vinylinfo.org/>), the vinyl industry in the US employs more than 100 000 workers. It is estimated that more than 15 000 tons of methyltin stabilizers are used each year in the US, and the use is growing annually (Kroschwitz and Howe-Grant, 1997; Leaversuch, 1999; Dobson *et al.*, 2005). Methyltin contaminants can now be found in various environmental media and ecosystems. Chau *et al.* (1997) reported that organotins, including methyltin compounds (e.g. MMT, DMT and TMT), were detected in surface waters and sediments throughout Canada (Chau *et al.*, 1997). It is interesting to note that TMT was found in industrial effluents and leachates from landfills even if DMT and MMT were absent (Chau *et al.*, 1997). Moreover, these methyltin contaminants were detected at levels up to 0.38 $\mu\text{g l}^{-1}$ in a drinking water reservoir and at levels of 0.02 to 0.50 $\mu\text{g l}^{-1}$ in the river downstream from the reservoir (Liu *et al.*, 2003). The levels of MMT and DMT in residential drinking water supplied by PVC pipes could be up to 0.29 and 0.05 $\mu\text{g l}^{-1}$, respectively (Sadiki and Williams, 1999). In addition, methyltin compounds were also found to be widely distributed in the marine environment (Shawky and Emons, 1998), suggesting that methyltin compounds are readily bioavailable in aquatic species (Fent, 1996).

A previous rat study has indicated that acute TMT exposure may compromise renal function (Robertson *et al.*, 1987). However, this result has been over-shadowed by the focus on the neurotoxicity of TMT. Our study was the first animal study designed to examine the adverse health effects associated with chronic TMT exposure, specifically the association between chronic exposure and the development of kidney stones. In the present study, we administered TMT for 180 days in the drinking water to healthy rats, which resulted in a cumulative dose that approximates 0, 0.1, 0.4 and 1.8-fold of the acute LD₅₀ dose, respectively. Two rats died during the treatment period in the highest dose group. Behavioral changes were also observed in the higher dose groups, but only during about the first 2 weeks of the exposure. There were no significant changes in the body weight of rats during the entire study period between the exposure groups and the control group and among the exposure groups. Together, the results suggest that the daily chronic doses of TMT are relative low and have minimal overt toxic effects on the rats.

Renal H⁺/K⁺-ATPase is important for the regulation of urinary H⁺ and K⁺ concentration. Previously, we reported that TMT exposure can directly inhibit the H⁺/K⁺ ATPase activity both in vitro and in vivo (Tang *et al.*, 2010). In human poisoning cases of TMT, symptoms of hypokalemia and alkaline urine have been reported (Tang *et al.*, 2008, 2010). The present rat study confirms that chronic low level TMT exposure significantly inhibits the activity of renal H⁺/K⁺-ATPase, which resulted in a dose-dependent increase in urinary pH. This is

relevant as alkaline urine favors calcium- and phosphate-containing stone crystallization (Coe et al., 2005; Moe, 2006). Compared with rats in the control group, rats with TMT exposure were found to have more wide spread calcium dispositions in the kidneys, which is a well-established indicator for kidney stone formation. While no kidney stones were identified in control and the lowest TMT dose group, rats in higher dose groups had stones in the kidney and urinary tract. XRD analysis also showed that kidney stones were mainly composed of struvite in addition to calcium-containing components. Struvite stones have been commonly associated with urinary tract infections (Coe et al., 2005). In addition, struvite stones can occur when urine pH rises to neutral or alkaline levels. It is not known whether the struvite stones found in the TMT-treated rats were solely as a result of the specific effect of H^+/K^+ -ATPase inhibition and renal tubular acidosis. If renal tubular acidosis was the only issue, calcium phosphate might be expected as the major components of stones. But given the fact that struvite is the major component, it is likely that other mechanisms in addition to alkaline urinary pH are involved in the stone formation with TMT exposure. We have observed cell necrosis and inflammation in the kidney of rats with TMT treatment. Further study is warrant to examine if TMT exposure may increase the risk of infection and inflammation, which could partially play a role in nephrotoxicity and the formation of struvite stones induced by TMT exposure.

Another interesting finding is that the uterus was significantly smaller in all TMT exposure groups compared with the control rats. To the best of our knowledge, there is no study that has investigated the reproductive and developmental toxicity of TMT. However, we found two studies that have examined the reproductive/developmental toxicity of DMT and MMT, respectively (Noda, 2001; Dobson *et al.*, 2005). Noda (2001) reported that there was a dose-dependent reduction of the maternal thymus weight in Wistar rats exposed to DMT. At the same dose range, DMT exposure was associated with a low fetal body weight, increased fetal mortality and anatomical defects (Noda, 2001). Other organotin compounds, e.g. butyltins, have been widely studied and are known to cause reproductive and developmental toxicity (Dobson *et al.*, 2005). The unexpected finding of reduced uterine weight with chronic TMT exposure warrants further investigation and suggests that TMT has the potential to elicit reproductive and developmental toxicity.

The present study has several limitations, notably, that while the daily TMT doses were low and resulted in little overt toxicity in rats, the level of exposure was still higher than most occupational exposures. Moreover, the study was designed to examine the relationship between TMT exposure and the risk of forming kidney stones. The study was not able to conduct a more thorough investigation of other markers of stone formation or markers of renal injury, such as crystalluria and urinary infection. Although our results support the conclusion that the inhibition of H^+/K^+ -ATPase by TMT may be a causative reason for the increased incidence of struvite stones, the conclusion is by no means definitive.

Conclusion

The findings from this study further demonstrate the association between chronic TMT exposure and an increased risk of developing kidney stones. Our results also suggest that the inhibition of H^+/K^+ -ATPase by chronic TMT exposure and a subsequent increase of urinary

pH at least partially contribute to the formation of struvite stones in the kidney and urinary tract. The wide spread use of methyltin compounds and their environmental contamination heightens concern regarding their nephrotoxic effects in workers and in the general public.

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Figure 1.

Drinking water consumption of rats during 180 days of trimethyltin (TMT) exposure. The consumption of drinking water of rats in the highest dose group, $131.25 \mu\text{g kg}^{-1} \text{ day}^{-1}$, was affected by TMT treatment ($P\text{-trend} < 0.05$).

Figure 2. Body weight of rats during 180 days of trimethyltin (TMT) exposure. Rats consistently gained body weight during the 180-day study period. No changes in body weight were observed in the TMT treatment groups when compared with rats in the control group.

Figure 3. Effects of chronic trimethyltin (TMT) exposure on the activity of renal H⁺/K⁺-ATPase. After 180-days TMT exposure, renal activities of H⁺/K⁺-ATPase are significantly decreased in a dose-dependent manner ($P\text{-trend} < 0.05$).

Figure 4.

Pathological changes of kidney and the stone formation in kidney/urinary tract post chronic trimethyltin (TMT) exposure. Pathological analyzes of the hematoxylin and eosin-stained kidney sections of rats from the vehicle control (A to C) and from chronic TMT exposure ($131.25\mu\text{g kg}^{-1}\text{ day}^{-1}$) (D to H): renal glomerulus with normal morphology (A), normal renal tubule (B), normal renal pelvis without sign of regenerating epithelial cells (C), calcium deposition in renal tubule (D), dilated lumen with flattened epithelial cells and interstitial edema (E), regeneration of epithelial cells (F), protein deposition (G) and inflammation (H). Gross anatomical analyzes shows that the stones were formed in kidney and in urinary tracts of rats treated with TMT for 180 days (I and J). (Images A, C, D, F, G and H were at a magnification of $400\times$, and B and E were $40\times$).

Table 1

Trimethyltin (TMT) concentration in urine and blood ($\mu\text{g ml}^{-1}$)

TMT ($\mu\text{g kg}^{-1}\text{ day}^{-1}$)	90 th day			180 th day		
	Urine		N	Whole blood		Red blood cells ^a
					Plasma ^a	
0	11	0	11	0	0	0
8.20	11	0.05 $\pm$ 0.01	10	0.05 $\pm$ 0.02	2.26 $\pm$ 0.43	0.02 $\pm$ 0.01
32.8	11	0.13 $\pm$ 0.06	10	0.17 $\pm$ 0.04	9.54 $\pm$ 1.75	0.11 $\pm$ 0.03
131.3	10	0.29 $\pm$ 0.14	8	0.41 $\pm$ 0.34	37.65 $\pm$ 22.20	0.46 $\pm$ 0.31

^a 0.5 ml of plasma and red blood cells was isolated from 2ml of whole blood and used for the analysis.

Table 2

Trimethyltin (TMT) exposure (designed vs. actual dose) over the 180-day exposure period

TMT ($\mu\text{g kg}^{-1}$ day^{-1})	Daily intake of TMT from drinking water ($\mu\text{g kg}^{-1}$ day^{-1})		Cumulative intake of TMT over the 180-day exposure period (fold of acute LD_{50} ^a)	
	Designed	Actual	Designed	Actual
0	0	0	0	0
8.20	8.20	9.07	0.10	0.11
32.8	32.8	34.7	0.41	0.43
131.3	131.3	138.6	1.66	1.77

^a Acute, single dose $\text{LD}_{50} = 14.7\text{mgkg}^{-1}$.

Table 3

Relative organ weight after 180 days of trimethyltin (TMT) exposure

Tissue/Organ	Control	8.20 ($\mu\text{g kg}^{-1} \text{ day}^{-1}$)	32.8 ($\mu\text{g kg}^{-1} \text{ day}^{-1}$)	131.3 ($\mu\text{g kg}^{-1} \text{ day}^{-1}$)
Heart	0.28 ± 0.02	0.32 ± 0.09	0.30 ± 0.03	0.29 ± 0.02
Liver	3.07 ± 0.29	3.12 ± 0.26	3.11 ± 0.46	3.29 ± 0.26
Pancreas	0.17 ± 0.02	0.17 ± 0.02	0.19 ± 0.03	0.18 ± 0.03
Lung	0.38 ± 0.06	0.40 ± 0.06	0.43 ± 0.05	0.41 ± 0.05
Kidney	0.54 ± 0.09	0.56 ± 0.11	0.55 ± 0.10	0.65 ± 0.22
Brain	0.54 ± 0.05	0.56 ± 0.07	0.54 ± 0.05	0.53 ± 0.06
Adrenal Gland	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Ovarian	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Uterus *	0.85 ± 1.00	0.45 ± 0.32	0.41 ± 0.51	0.25 ± 0.10
Thymus Gland	0.08 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.02

Value represents the weight of organ $\div$ body weight $\times$ 100 (mean $\pm$ SD).* $P < 0.05$ relative to the control.

Table 4

Renal function analysis in rats after 90 and 180 days of trimethyltin (TMT) exposure

TMT ($\mu\text{g kg}^{-1}\text{day}^{-1}$)	24-h urine volume (ml)		Specific gravity (g ml^{-1})		Urinary creatinine ($\mu\text{mol l}^{-1}$)		pH	
	90 th day	180 th day	90 th day	180 th day	90 th day	180 th day	90 th day	180 th day
0	15.77 $\pm$ 6.92	15.41 $\pm$ 7.00	1.020 $\pm$ 0.006	1.010 $\pm$ 0.005	319.75 $\pm$ 99.07	342.3 $\pm$ 65.57	6.67 $\pm$ 0.31	6.99 $\pm$ 0.42
8.20	19.82 $\pm$ 4.60	17.86 $\pm$ 5.35	1.019 $\pm$ 0.004	1.012 $\pm$ 0.005	298.33 $\pm$ 64.61	277.00 $\pm$ 75.67	6.89 $\pm$ 0.34	6.94 $\pm$ 0.37
32.8	21.45 $\pm$ 6.71 *	25.10 $\pm$ 9.76 *	1.019 $\pm$ 0.006	1.015 $\pm$ 0.005	221.91 $\pm$ 58.73 *	209.50 $\pm$ 80.67 *	7.29 $\pm$ 0.44 *	7.20 $\pm$ 0.38 *
131.3	33.38 $\pm$ 12.45 *	35.72 $\pm$ 15.32 *	1.016 $\pm$ 0.002	1.011 $\pm$ 0.006	125.02 $\pm$ 68.48 **	178.10 $\pm$ 84.52 *	7.45 $\pm$ 0.40 *	7.42 $\pm$ 0.32 *

Value represents the mean $\pm$ SD. Significantly different from the control* $P < 0.05$ ** $P < 0.01$.

Gross anatomic and histologic analysis of kidneys in rats after a 180-day exposure to trimethyltin (TMT)

Table 5

Symptoms	TMT ($\mu\text{g kg}^{-1} \text{ day}^{-1}$)			
	Control	8.20	32.8	131.3
Gross anatomic analysis^a				
Stone in kidney/urinary tract [*]	0 (0)	0 (0)	1 (10.0)	3 (33.3)
Histology analysis^b				
Number of kidney examined (left & right side)	n=22	n=22	n=22	n=22
Calcium deposits	10 (45.5)	14 (63.6)	12 (54.6)	10 (55.6)
Epithelial cell proliferation	0 (0)	0 (0)	0 (0)	4 (22.2)
Protein deposition	0 (0)	0 (0)	0 (0)	2 (11.1)
Renal tubular dilation	0 (0)	0 (0)	2 (9.1)	6 (33.3)
Inflammatory infiltration	2 (9.1)	0 (0)	2 (9.1)	4 (22.2)

^{*} Trend t-test

^a Number of rats with kidney/urinary tract stones (percentage of responding)

^b Number of kidneys with specified pathological changes (percentage of responding).