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Levels of Tin and Organotin Compounds in Human Urine Samples from Iowa, United States

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Abstract

Exposure to tin in the general US population is near ubiquitous, as determined using urinary tin levels measured by inductively coupled plasma mass spectrometry (ICP-MS). Urinary tin levels are associated with chronic health outcomes, such as diabetes; however, it is unclear if these associations are due to the presence of inorganic and organic forms of tin in urine. To address this knowledge gap, levels of total tin and several organotin compounds (OTCs) were measured in convenience urine samples from pregnant women and adults from Iowa, United States. Total tin and OTC levels in urine samples were quantified using ICP-MS and gas chromatography with pulsed flame photometric detection (GC-PFPD), respectively. ICP-MS detected tin in almost all urine samples from both study populations. Low levels of dibutyltin were detected in two out of fifty human urine samples. Importantly, storage of urine samples in plastic containers, but not HNO₃-pretreated glass vials drastically reduced the recoveries of OTCs, in particular, tributyltin. Although their detection frequency is low, exposures to OTC should be considered when studying associations between human exposures to tin compounds and adverse health outcomes; however, urinary OTC levels measured in banked urine samples may not be suitable as biomarkers of OTC exposure.

Keywords

dibutyltin; inorganic tin; monobutyltin; organotin speciation; sample storage; tetrabutyltin; tributyltin; urinary tin

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Introduction

Tin is not a non-essential metal in humans and has been detected in both inorganic and organic forms in the environment, wildlife, and humans. ^[1] Inorganic forms of tin are used, for example, in tin-coated food and beverage cans to prevent corrosion; in soft solders, bronzes or other alloys; for the manufacturing of electrically conducting films; and to produce flat window glass surfaces. Stannous chloride (SnCl_2) is a food additive approved by the U.S. Food and Drug Administration. ^[2] Organotin compounds (OTCs) were used as stabilizers for polyvinyl chloride (PVC), biocidal textile finishes, catalysts for the synthesis of polyurethanes and silicones, agricultural fungicides, antifouling agents for ships, and in glass coatings. ^[3, 4] Because of their uses, OTCs can be found in sediments and other environmental compartments. ^[5, 6] The production and use of organotin compounds for many applications, in particular as antifouling agents, are now banned because of environmental concerns. ^[3, 7] Besides, chemical and biochemical methylation can result in the conversion of inorganic tin into the OTCs in the environment. ^[1] Some methyltin compounds, such as dimethyl and trimethyl tin, occur naturally and are formed by bacterial methylation of inorganic tin. ^[8, 9] Both inorganic and organic forms of tin are released into the environment from natural and anthropogenic sources, thus resulting in the potential for human exposures.

Humans are exposed to inorganic tin by ingestion of contaminated food or drinking water, inhalation or dermal absorption. ^[1, 10, 11] The consumption of canned foods is thought to make a significant contribution to the daily intake of inorganic tin; however, some human biomonitoring studies do not report a positive relationship between dietary tin levels and urinary tin levels, possibly because of the complex factors affecting tin absorption and elimination. ^[12-16] The diet, in particular, seafood, is the primary route of human exposure to OTCs. ^[17-20] A recent finding suggests that the estimated daily intake of OTCs is below the tolerable daily intake. ^[21]

Tin was detected in the urine of 87.05% of adults and 91.29% children in the United States using ICP-MS. ^[22] Tin is also present at low levels in other human tissues. ^[23] Although human exposure to tin compounds is nearly ubiquitous, the adverse health effects of exposure to this non-essential element are poorly characterized. Depending on the chemical form of the inorganic tin compound, consumption of food products contaminated with tin can result in acute gastrointestinal illnesses. ^[1, 11] A few epidemiological studies suggest that environmental exposure to tin may be associated with chronic health outcomes, such as diabetes. ^[24-27] OTCs exposure has been linked by epidemiological studies to adverse developmental outcomes, ^[28, 29] and animal studies suggest that some OTCs, in particular, tributyltin (TBT), are environmental obesogens. ^[30-32] However, more research is needed to better characterize human exposures to inorganic and organic forms of tin and identify potential adverse health outcomes associated with these exposures.

Urine is a frequently used matrix for human biomonitoring studies, including studies of human tin exposures. Inorganic tin is primarily excreted with the feces and urine in humans following oral exposure. ^[16, 33] Similarly, animal studies demonstrate that absorbed

inorganic tin is eliminated with the feces (via the bile) or urine. [34, 35] Like inorganic forms of tin, OTCs are also eliminated with the feces, bile or urine in a compound specific manner. [1] Animal studies suggest that OTCs are excreted with the urine of humans. However, it is unknown whether or not OTCs contribute to total urinary tin levels in humans, which in turn could explain associations between urinary tin levels and adverse health outcomes. To close this knowledge gap, this study measured levels of urinary tin and OTCs, in particular, monobutyltin (MBT), dibutyltin (DBT), TBT and tetrabutyltin (TeBT), in convenience urine samples from two study populations from Iowa, United States.

Materials and methods

Chemicals and materials

An analytical standard mixture containing chlorides of MBT, DBT, TBT, and TeBT was obtained from Accustandards Inc. (New Haven, CT, USA). Tetraethyltin (TET, surrogate recovery standard) was obtained from Alfa Aesar (Tewksbury, MA, USA). Dibutyltin dichloride-d₁₈ (DBT-d₁₈, surrogate recovery standard), tributyltin chloride-d₂₇ (TBT-d₂₇, surrogate recovery standard), and tetrabutyltin-d₃₆ (TeBT-d₃₆, surrogate recovery standard) were provided by Toronto Research Chemicals (North York, Canada). Tetraphenyltin (TPhT, internal standard or volume corrector) was obtained from TCI America (Portland, OR, USA). Hexane and methanol were pesticide grade and obtained from Fisher Scientific (Hanover Park, IL, USA). ICP-MS grade multi-element standard containing inorganic tin (600 mg/L in 5% HNO₃, 1% HF, and 0.5% HCl) was obtained from High Purity Standards (Charleston, SC, USA). Trace metal grade nitric acid was also purchased from Fisher Scientific. Sodium acetate anhydrous and sodium chloride ACS grade were both obtained from Research Products International (Mt. Prospect, IL, USA). Sodium tetraethyl borate (97% purity) was provided by Sigma Aldrich (St. Louis, MO, USA). To eliminate the issue of analyte adsorption onto the glassware, all glassware used were soaked in 2% nitric acid solution overnight. [36] The glassware was thoroughly rinsed with hot tap water followed by deionized water, air dried and baked at 325 °C for 6 h.

Effect of storage container, temperature and storage time on OTCs recoveries

The effect of storage container and temperature on the recovery of the target analytes was explored to simulate storage conditions potentially utilized in human biomonitoring studies. Spiked urine samples for these experiments were prepared by adding the 1.25 mL of the OTC standard (200 ng/mL in methanol) into 48.75 mL pooled urine sample to afford nominal concentrations of each analyte of 5 ng/mL. Two mL aliquots of this urine sample were stored in plastic urine specimen vials (Fisher catalog # 12-567-502), plasma specimen vials (Fisher catalog# 50-809-242), glass vials (Fisher catalog # 03-391-36) or polypropylene vials (Fisher catalog # 50-121-5200). Aliquots were stored at the different storage temperatures (4 °C, -20 °C and -80 °C) for a maximum of 4 days before the OTCs analysis. In a separate experiment, the effect of storage time on the recoveries of OTCs was assessed with a pooled urine sample (97.5 mL) spiked with 2.5 mL of the butyltin chloride standard (200 ng/mL in methanol) to afford a nominal OTCs concentration of 5 ng/mL. Aliquots (2 mL) of the spiked urine sample were stored at -20 °C in pretreated glass vials

(Fisher, catalog # 1495935A) and analyzed after storage for 1 to 84 days to assess the stability of the OTCs in human urine.

Study participants and sample collection

Two biomonitoring studies were performed to determine if OTCs (*i.e.*, MBT, DBT, TBT, and TeBT) contribute to the urinary tin levels in humans and if urine levels of OTCs are affected by the material of the storage container. Signed informed consent was obtained from all study participants. Urine samples from pregnant women (N=20) were collected by staff from the Maternal Fetal Tissue Bank (MFTB) at the University of Iowa in excess from clinically-indicated urine analyses at outpatient prenatal appointments (IRB# 200910784). Details describing the MFTB have been described previously. [37] These urine samples were stored at 4 °C in the clinic for 1 to 4 hours before being transported to the laboratory on wet ice. Samples were then aliquoted, snap frozen, and stored at –80 °C. Samples for this project were randomly chosen from the MFTB sample pool. For the second study, fresh urine samples were collected in spring 2018 from a total of 30 adult individuals (IRB# 201408810). These urine samples were collected in clean, HNO₃ pretreated glass jars and immediately aliquoted into glass vials (Fisher, catalog # 1495935A) or plastic vials (Fisher catalog # 12–567-502). These samples were stored at –20 °C for a maximum of 4 days before OTC analysis.

Derivatization and extraction of OTCs from urine

Organotin speciation in urine was conducted following a published protocol with some modifications. [38] Briefly, 2 mL aliquots of urine were placed into pretreated glass vials and weighed to determine the mass of the urine sample. Surrogate recovery standards (TET in hexane, DBT-d₁₈ in methanol, TBT-d₂₇ in methanol and TeBT-d₃₆ in hexane, 10 ng each) were added to each urine sample, followed by addition of 1 mL sodium acetate buffer solution (1 M, pH 4.5) and 0.2 g sodium chloride. Samples were vortexed until the sodium chloride was dissolved, followed by derivatization with 1 mL of sodium tetraethyl borate (1% methanolic solution) for 1 h at room temperature. Sodium tetraethyl borate was selected as derivatization reagent because it is easier to handle compared to other, more reactive derivatization reagents, such as propyl magnesium bromide. The samples were extracted twice with 5 mL of hexane, and the hexane extracts were transferred into another pretreated glass vial. The extract was reduced to approximately 200 µL under a gentle stream of nitrogen and transferred into a GC vial. The internal standard (volume corrector), TPhT (15 ng in hexane), was added to each GC vial before analysis. Urine blanks and urine samples spiked with the target analytes (MBT, DBT, TBT and TeBT, 5 ng per mL sample in methanol) were analyzed with each sample set.

Analysis of OTCs

The OTCs analysis was performed using an Agilent 7890A gas chromatograph equipped with an Agilent 7693 autosampler and a pulsed flame photometric detector (PFPD model 5383; OI Analytical, College Station, TX, USA). A SPB-1 column (30 m length; 0.25 mm inner diameter; 1.0 µm film thickness; Supelco, Bellefonte, PA, USA) was used for all analyses. The injector operated in the splitless mode at 250 °C. The detector temperature

was 320 °C. The hydrogen, air 1 and air 2 (makeup) flows for the detector were 14 mL/min, 12 mL/min, and 10 mL/min, respectively. The oven temperature program was as follows: 100 °C for 0 min, then 15 °C/min to 150 °C, hold for 5 min, 0.5 °C/min to 160 °C, hold for 0 min, 1 °C/min to 165 °C, 20 °C/min to 280 °C and hold for 20 min. Helium was used as the carrier gas with a flow rate of 2.5 mL/min. Relative retention times of all analytes were within 0.5% of the average relative retention time of the reference standard. [39] The concentration of OTCs in each sample was calculated using the internal standard method based on their relative response factors. The concentrations of the target analytes were adjusted using the recoveries of spiked urine samples analyzed in parallel.

Quality assurance/quality control in OTCs analysis

The detector response was linear over the range 0.5–250 ng/mL for all analytes, including recovery and internal standards ($R^2 > 0.99$). The method limit of detection (LOD) was calculated from the calibration curves following a guideline from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. [40] The LOD was calculated from the formula; $LOD = 3.3 \frac{\sigma}{S}$, where σ is the standard deviation of the 12 urine blank measurements and S is the slope of the calibration curve. The LODs determined by this method were 1.9 ng/g urine, 0.4 ng/g urine, 0.4 ng/g urine and 0.4 ng/g urine for MBT, DBT, TBT, and TeBT respectively. The mean analytical recoveries of the recovery standards were $83 \pm 11\%$ (range: 64% to 110%; $n = 153$), $77 \pm 13\%$ (range: 59% to 104%; $n = 153$), $82 \pm 11\%$ (range: 65% to 109%; $n = 153$) and $86 \pm 11\%$ (range: 62% - 123%; $n = 153$) for TET, DBT-d₁₈, TBT-d₂₇ and TeBT-d₃₆ respectively. Mean recoveries of the target analytes in spiked urine samples were $93 \pm 19\%$ (range: 56% to 126%; $n = 43$), $78 \pm 13\%$ (range: 56% to 117%; $n = 43$), $85 \pm 12\%$ (range: 65% to 108%; $n = 43$) and $86 \pm 11\%$ (range: 55% to 100%; $n = 43$) for MBT, DBT, TBT and TeBT, respectively.

Total urinary tin analysis

Pooled human urine samples were used to assess the effect of storage condition and time on total urinary tin concentrations. In short, 48.75 mL of a pooled urine sample was spiked with 1.25 mL of ICP-MS grade inorganic tin standard (200 ng/mL freshly prepared in water) to afford nominal concentration of 5 ng/mL. One mL aliquots were transferred into different containers and stored at -20 °C for 24 h before analysis or in polypropylene tubes for a period of 1 to 84 days for the stability study. Concentrations of total tin in urine samples were measured using ICP-MS at the State Hygienic Laboratory (Ankeny, IA, USA).

Intermediate calibration standards were prepared from a commercially available stock standard in 2% HNO₃, 1% HCl. Working calibration standards were prepared by adding 0.5 mL of the intermediate standard to 2.0 mL of the carrier (2% HNO₃, 0.002% Triton X-100, 10 µg/L Rhodium as internal standard) and 0.5 mL of base urine (< 3 µg/L Sn). Urine samples were prepared for analysis by adding 0.5 mL of sample to 2.0 mL of the carrier and 0.5 mL of calibration blank. An aqueous calibration blank was analyzed before the samples to avoid subtraction of any tin content in the base urine from the sample result. This aqueous blank was identical to the calibration blank except that 0.5 mL of reagent water was used in place of the base urine. Calibration was verified at the reporting level and mid-calibration

range. Reference material urinary tin samples were acquired from proficiency testing provider Centre de toxicologie du Québec (Québec, Canada). The reference materials were analyzed at the beginning and end of the run to establish instrument stability throughout the analysis. The reference material also served as a second-source verification of the calibration standards. Analysis of total tin in the urine samples was conducted using ICP-MS (NexION 350D; Perkin Elmer, Waltham, MA, USA). The LOD for the total tin analysis by ICP-MS was 0.15 µg/L.

Results and discussion

Effect of storage conditions on the recoveries of OTCs in human urine

Urine samples from epidemiological studies are typically stored in plastic tubes. We, therefore, investigated how storage conditions affect the recoveries of our target analytes from human urine. Our results indicate that the storage of the urine samples has a critical impact on OTC measurements (Figure 1). Specifically, a significant reduction of TBT (> 70%) was observed in spiked pooled human urine samples stored for only 24 h at –20 °C in plastic plasma tubes, urine tubes, and polypropylene tubes compared to HNO₃-treated glass vials. In contrast, > 80% of MBT, DBT, and TeBT were recovered from the same samples irrespective of the storage container used.

In separate experiments, the effect of storage temperature on the recoveries of OTCs was evaluated for pooled human urine samples stored in plastic urine tubes vs. HNO₃-treated glass vials at 4 °C, –20 °C and –80 °C (Figure 2). The recoveries of the OTCs were comparable for the storage temperatures investigated, both for spiked urine samples stored in plastic tubes and HNO₃-treated glass vials. Lower storage temperature did not improve the poor recoveries of TBT from urine samples stored in plastic tubes.

An assessment of the stability of OTCs in urine samples in HNO₃-treated glass vials showed no appreciable change of OTC levels in spiked urine samples stored over 84 days at –20 °C (Figure 3). Our stability results are consistent with an earlier study which indicates that OTCs (*i.e.*, MBT, DBT, TBT, TeBT, heptyltin, diheptyltin, octyltin, dioctyltin, tricyclohexyltin and triphenyltin) are stable in water for up to 20 days.^[41] These findings suggest that urine samples for OTC analyses are ideally collected in HNO₃-treated glass vials.

Effect of storage conditions on the recoveries of total urinary tin in human urine

Storage time (up to 84 days) and storage container did not affect total urinary tin concentrations in spiked urine samples, as determined by ICP-MS (Figure 4). In contrast to TBT, storage of spiked urine samples in plastic tubes vs. HNO₃-treated glass vials did not affect the total urinary tin concentrations. These observations are not surprising because urinary tin levels are routinely determined by ICP-MS as part of human biomonitoring studies, including the National Health and Nutrition Examination Survey (NHANES).

Urinary levels of tin and OTCs in Iowa, United States

Total tin and OTC levels were measured by ICP-MS and GC-PFPD in convenience urine samples from pregnant women (N=20; Table 1) as well as adult volunteers from Iowa (N=30; Table 2). The first set of samples was obtained from the MFTB at the University of Iowa and stored in plastic vials at -80°C . Because levels of OTCs, in particular, TBT, decrease quickly in urine samples stored in conventional plastic vials, we also collected urine samples from 30 adult volunteers living in Iowa and stored aliquots of each sample under identical conditions either in conventional plastic vials or HNO_3 -treated glass vials. The goal of this study was to assess if storage in HNO_3 -treated glass vials improved detection frequencies of OTCs.

Tin was detected in all samples collected from pregnant women by ICP-MS (Table 1). The geometric mean and median level of urinary tin in this study population was 0.21 ng/mL and 0.20 ng/mL, respectively. Tin was also detected in 97% of the adult human urine samples (Table 2). The median and geometric mean urinary tin levels found in the second study population were 0.53 ng/mL and 0.57 ng/mL respectively. It is noteworthy that urinary tin levels appear to be lower in pregnant women compared to the adult study population. This preliminary observation raises the question if there are differences in exposure or physiological effects that explain these differences in urinary tin levels.

The median and geometric mean urinary tin levels in the convenience samples from the adult Iowa population (Table 2) are comparable to tin levels reported for US adults participating in NHANES 2011–2014, with median and geometric mean levels of 0.42 ng/mL and 0.49 ng/mL. [22] For comparison, geometric mean urinary tin levels reported in biomonitoring studies conducted in 2010 to 2011 in Belgium were 0.289 ng/mL (range 0.26 – 0.32 ng/mL, N= 1022). [42] Compared to the present study, higher geometric mean levels of urinary tin were reported in an adult population from Germany in 2005 (0.84 ng/mL, range 0.06 – 204 ng/mL, N=87); in Japanese women in 2007 (0.99 ng/mL, N=37) and an earlier NHANES study (3.13 ng/mL, N=496, 1988 to 1994) respectively. [12, 43-45]

Only DBT was detected in two out of twenty samples obtained from pregnant women. Briefly, the urine sample from donors #3 and #5 contained 0.7 ng/g urine and 3.7 ng/g urine of DBT, respectively. Levels of other OTCs were below the detection limit in all samples. Importantly, no OTCs were detected in samples from the second study population, irrespective of the storage material (i.e., plastic vs. HNO_3 -treated glass vials). These findings suggest that the individuals investigated in this study may not be exposed to OTCs. Alternatively, urine may not be an appreciable route of OTC elimination in humans.

Conclusions

Consistent with findings from the US general population participating in NHANES 2011–2014, tin was detected by ICP-MS in almost all urine samples analyzed as part of this study, with urinary tin levels being comparable to other human biomonitoring studies. Only DBT was detected by GC-PFPD in two out of fifty human urine samples. Thus, OTCs typically do not contribute to the total urinary tin in most samples analyzed. While the material of the

storage container did not affect recoveries of tin determined by ICP-MS, plastic storage containers drastically reduced the recoveries of OTCs, in particular, TBT. These results indicate that banked urine samples are not suitable for studies of adverse health outcomes associated with OTC exposure because these samples are typically stored in plastic vials. Because of their well-documented obesogenic effects, OTCs should be considered as a factor contributing to adverse health outcomes associated with exposures to different forms of tin, despite their low detection frequencies in this study.

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Figure 1.

Recoveries of TBT from human urine samples were lower from samples stored in different glass or plastic containers. Aliquots of a pooled human urine sample containing MBT, DBT, TBT, and TeBT (5 ng/g of each OTC) were stored at -20°C for 24 h before analysis, see Materials and Methods for additional details

Figure 2.

Recoveries of OTCs from human urine samples were not affected by storage temperature, both for samples stored in (A) HNO₃ pretreated glass vials or (B) urine tubes. Aliquots of a pooled human urine sample containing MBT, DBT, TBT, and TeBT (5 ng/g of each OTC) were stored at 4 °C, -20 °C, and -80 °C for 4 days before analysis, see Materials and Methods for additional details. Concentrations were normalized to the concentration of samples analyzed on day 1

Figure 3.

Concentrations of (A) MBT, (B) DBT, (C) TBT and (D) TeBT in human urine samples do not change with storage times. Aliquots of a spiked urine sample were stored in clean, HNO_3 pretreated glass vials at -20°C for up to 84 days. Concentrations were normalized to the concentration of samples analyzed on day 1

Figure 4.

Total urinary tin concentrations, determined by ICP-MS, were not affected by (A) storage times of up to 84 days or (B) storage in plastic vs. glass storage container at -20°C for 24 h. For the time course experiment, aliquots of spiked pooled human urine samples (5 ng/mL inorganic tin) were stored in polypropylene (PP) tubes at -20°C for up to 84 days to assess if storage time affects total urinary tin concentrations

Table 1.

Total urinary tin levels in urine samples from pregnant women from Iowa. Samples were obtained from the Maternal Fetal Tissue Bank of the University of Iowa

Donor #	Total urinary [Sn] ng/mL	Donor #	Total urinary [Sn] ng/mL
1	0.52	11	0.36
2	0.40	12	0.16
3	0.31	13	0.20
4	0.32	14	0.09
5	1.03	15	0.09
6	0.24	16	0.13
7	0.19	17	0.16
8	0.08	18	0.11
9	0.07	19	0.30
10	0.11	20	0.55

Table 2.

Total urinary tin in urine samples collected in spring 2018 from male and female adults in the Iowa City, Iowa area

Donor #	Total urinary [Sn] ng/mL	Donor #	Total urinary [Sn] ng/mL	Donor #	Total urinary [Sn] ng/mL
1	0.53	11	0.50	21	0.74
2	1.10	12	0.17	22	0.24
3	0.35	13	0.27	23	1.30
4	0.34	14	1.00	24	0.23
5	0.71	15	0.79	25	not detected
6	1.00	16	0.23	26	0.63
7	1.10	17	0.43	27	0.45
8	1.50	18	0.40	28	0.66
9	0.76	19	0.35	29	0.44
10	2.10	20	1.20	30	0.38