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Tin Compounds and Major Trace Metal Elements in Organotin-Poisoned Patient's Urine and Blood Measured by Gas Chromatography-Flame Photometric Detector and Inductively Coupled Plasma-Mass Spectrometry

Jiang Gui-bin, Zhou Qun-fang, He Bin

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Post Office Box 2871, Beijing, 100085, China

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Organotin compounds, especially dimers and trimers, are widely used in industrial and agricultural as stabilizer, catalysts and biocides(Warren, 1973). Occasional organotin poisoning occurred from careless use in a worldwide scope. Elucidation of the biological effects and environmental impacts of tin species cannot be achieved by conventional total tin analysis but requires analytical techniques that allow both the identification and the quantitative determination of the variety of inorganic and organic forms(Dirkx, *et al.*, 1989). Over the last ten years, continuous progresses have been made in the development of analytical methodologies for the determination of such compounds(Cappon, 1988; Donard and Martin, 1992; Lobinski and Adams, 1993). These include a chromatographic separation(Matthias, *et al.*, 1986; Maguire, *et al.*, 1982; Maguire and Tkacz, 1983; Mueller, 1984) or "cold trapping" with subsequent boiling point separation(Valkirs, *et al.*, 1986; Braman and Tompinks, 1979; Hodge, *et al.*, 1979) of tin species, followed by atomic absorption, mass spectrometric or flame photometric detection. Because of the high separation power of GC columns, the availability of selective and sensitive detectors and the reduced requirement for hazardous solvents, the GC techniques are widely used. However, the ionic organotin compounds and the inorganic Sn(IV) in environmental and biological matrices generally have rather high boiling points; a derivatization step is required to lower their boiling-points to suit to GC separation. Hydride formation using $NaBH_4$ and alkylation using Grignard reagents or $NaBEt_4$ have been used for this purpose(Stab, *et al.*, 1992; Clark, *et al.*, 1987; Donard, *et al.*, 1986; Cai, *et al.*, 1993). These derivatization methods do not change the identities of the original compounds, which makes it possible to identify each of the tin species.

During 1999 New Year's Days, more than 1000 people in southeast China's Jiangxi province, Longnan and Dingnan county, were poisoned by misusing organotin contaminated industrial lard as cooking oil, among them, hundreds people were hospitalized and three people died from it. In this paper, we analyzed urine and blood samples from the patients in this incident. The trace levels of inorganic and organotin compounds were determined by a simple and sensitive method which was based on the conversion of tin species into corresponding pentylated compounds and subsequent analyzed by capillary GC-FPD. Total tin and 11 major trace metal elements were measured by ICP-MS. To our knowledge, this is the first time to found methyltin species in human urine and blood.

Correspondence to: Jiang Gui-bin

MATERIALS AND METHODS

A GC-9A gas chromatograph (Shimadzu, Japan) equipped with a capillary column (HP-1, 25m × 0.32mm i.d. × 0.17μm coating) was used throughout the experiment. The GC temperature was programmed from 50℃ (hold for 2min) to a final temperature of 200℃ at 10℃/min, and then hold for 5min. The injector temperature was 220℃. High purity nitrogen was used as the carrier gas and column head pressure was kept at 260 kPa. A laboratory-made flame photometric detector using quartz surface-induced tin emission(QSIL-FPD) was used to differentiate methyltin compounds, its configuration and application was reported previously(Jiang and Xu, 1996; Jiang *et al.*, 1996). The detector temperature was set at 160℃. Hydrogen and air were controlled at 260ml/min and 90ml/min. The measurement was carried out by using a 394nm interference filter. Chromatograms were recorded on a SC-1100 data processing system.

A Plasma-Quad 3 (VG Elemental, Winsford, UK) ICP-MS was used for the determination of total tin and 11 major trace elements. General instrumental operation conditions were given in Table 1.

Table 1. ICP-MS operation conditions

Forward power	1350w
Reflected power	<5w
Coolant argon flow rate	14 l/min
Auxiliary gas floe rate	0.9 l/min
Nebulizer gas flow rate	0.8 l/min
Sample uptake rate	1.0 ml/min
Sampling depth	15 mm
Mass spectrometer	
Sampler (nickel) orifice	1.0 mm
Skimmer (nickel) orifice	0.7mm
1st stage pressure	1.6×10^{5} mPa
2nd stage pressure	1.0×10 mPa
3rd stage pressure	1.7×10^{-1} mPa
Date acquisition	Range-scanning mode
Mass range	m/z 50-210
Total acquisition time	50 s

Trimethyltin chloride (TMT, 97%), dimethyltin dichloride(DMT, 97%) and monomethyltin trichloride(MMT, 97%) were obtained from Aldrich Chem. Co. (USA). Stock solutions was prepared by directly weighed suitable amount of standard compounds and dissolved in methanol to form a concentration level of 1mg/g (as Sn). Inorganic Sn(IV) solution was prepared by dissolving metal Sn in 6M HCl in a water bath controlled at 40°C. After the solid was completely dissolved, the solution was diluted with methanol to form a concentration of

4.0mg/ml (as Sn). Working standard solutions (10μg/ml) were prepared by diluting the stock solutions with de-ionized water with the pH adjusted to 2 using HCl to ensure the stability.

The extraction solution, which was prepared just before use, was obtained by dissolving tropolone (98%) (Acros Co., U.S.A) in cyclohexane to a final concentration of 0.1%.

The Grignard reagent of *n*-pentylmagnesium bromide (*n*-PeMgBr, 2.0M) was prepared in the laboratory using the standard synthetic methods(Zhou, 1999). The internal standard metyl-tri(*n*-prothyl)tin, $MeSn(n\text{-}Pr)_3$, was synthesized by reaction of $MeSnCl_3$ (10μg/ml, cyclohexane) with 2.0M *n*-PrMgBr.

Urine and blood samples were collected from the patients who was hospitalized during the food poisoning event.

For the sample preparation in GC-FPD analysis, 2ml of urine sample or 0.40 gram of the well-distributed whole blood sample was placed in a separatory funnel together with 5ml citric acid-NaH_2PO_4 buffer solution (pH5). 2ml of the internal standard ($MeSnPr_3$, 80ng/ml) and 2.5ml of 0.1% tropolone-cyclohexane were added in sequence. The mixture was extracted for 15 minutes in an ultrasonic bath. After removal of the supernatant, the residue was re-extracted once again with another 2.5ml portion of extraction solution. After 10min centrifugation at 2000r/min, the combined organic phases were dried on anhydrous Na_2SO_4.

Each extract was adjusted to 5ml with cyclohexane, and reacted with 0.5ml of 2.0 M *n*-PeMgBr for 15min in an ultrasonic bath. Then the excess Grignard reagent was destroyed by careful, dropwise addition of about 2ml of 0.5M H_2SO_4. An additional wash with about 60ml of de-ionized water followed. The organic layer was separated and the aqueous phase was reextracted with 5ml of cyclohexane. A 0.2 mg of anhydrous sodium sulfate was added to the combined organic layers in the vial to remove traces of water.

The samples were purified by Florisil (0.8mg) packed in a glass pipet and prewashed with 5ml of cyclohexane. Another 5ml of cyclohexane was used to elute the pentylated derivatives. The eluted solution was concentrated to 5ml under a stream of nitrogen. An aliquot was injected into GC for analysis.

For the sample preparation in ICP-MS analysis. A 0.5ml of urine or 1.5g of whole blood was put into a 30ml Teflon container and 2ml of concentrated HNO_3 was added. The container was then covered with Teflon and heated on a hot plate kept at ~50°C for 8hr. After the container cooled down to room temperature, the Teflon cover was removed and then another 0.5ml of HNO_3 and 1ml concentrated perchloric acid ($HClO_4$) were added in sequence. The container was heated again and the solution was evaporated until fumes of $HClO_4$ nearly disappeared. The residue was dissolved in a small amount of 0.01M HNO_3. The solution was

transferred into a 10ml volumetric flask with the addition of 20ppb Indium as internal standard and diluted to the mark with 0.01M HNO_3 to obtain the final solution of 10 ng/mL.

RESULTS AND DISCUSSION

Because the Grignard reagent would be destroyed by active H^+, the organotins in the aqueous samples couldn't undergo the pentylation directly. It was necessary to change the interested compounds into organic solvents, which didn't contain active H^+. According to the physicochemical properties of all organotins involved, there was obvious tendency for higher polarity and water solubility of organotin compounds with fewer and shorter alkyl chains attached to the tin atom. Therefor it was impossible to extract all organotins efficiently by an organic solvent alone. Tropolone was well known to form metal complexes which could be easily extracted by organic solvents(Forsyth, *et al.*, 1993). It could greatly help to increase the extraction recoveries of all organotin compounds. The pH of the sample solutions was adjusted to 5 by citric acid-NaH_2PO_4 buffer for the well extraction of organtins(Dirkx, *et al.*, 1989). In order to be able to separate the micro-volume of cyclohexane solutions from the sample layer, a separatory funnel equipped with a capillary neck was used(Cleuvenbergen, *et al.*, 1984).

Pentylation, obtained by using the Grignard reagent *n*-PeMgBr, was chosen for conversion of the various mono, di, and tri-substituted organotin compounds and inorganic tin into tetra-substituted compounds. Pentylation was preferred over other alkylation for the following reasons: in the natural environment, pentylated organotins rarely existed, while other alkylated tin compounds were familiar. For example, methylation of tin(IV) and butyltin species usually occurred leading to methyltins and mixed methylalkyltins (Guard, *et al.*, 1981; Hallas, *et al.*, 1982). Further methylation of these environmental metabolites in the derivation step would exclude the possibility of determining these conversion and degradation products. Furthermore, pentylation facilitates the separation of organotin derivatives using gas chromatography, as the order of elution followed increasing degree of substitution. The pentyl derivatives had a somewhat higher boiling point compared to other alkylated compounds, which reduced evaporative losses during concentration steps. A 15-min reaction time in an ultrasonic bath was found to be sufficient for the pentylation of various organotins.

GC column performance degraded greatly during chromatography of initial (uncleaned) extracts, resulting in peak broadening or tailing(Forsyth, *et al.*, 1993), so the purification step was necessary. In this study, a short florisil column pre-washed with cyclohexane was used. The results showed that the impurities could be effectively got rid of with little loss of the target compounds by the elution of 5ml of cyclohexane.

Analysis by GC with flame photometric detector using quartz surface-induced tin emission offers advantages over the hitherto reported methods in terms of

sensitivity and cost(Jiang and Xu, 1996; Jiang, *et al.*, 1996). Using HP-1 capillary column with a temperature programming, all of the interested compounds were baseline separated. Satisfying results were obtained owing to a combination of the detector's reliability and high sensitivity. In order to improve the precision of the measurement, methyltripropyltin($MeSnPr_3$) as internal standard was used. This compound was synthesized by preparing the Grignard reagent, *n*-PrMgBr, and reacted with monomethyltin chloride, $MeSnCl_3$. The reaction product was diluted to a suitable level and used throughout the whole process.

Compared with the GC-FPD figure of pentylated standard tin compounds, the urine samples contained tri-, dimethyltin and inorganic tin while blood sample contained trimethyltin and an unknown compound which was presumed as an organotin compound as its peak shape accorded with the character of organotin compounds. Fig.1 showed the GC-FPD figures comparison of the standards, urine and blood samples.

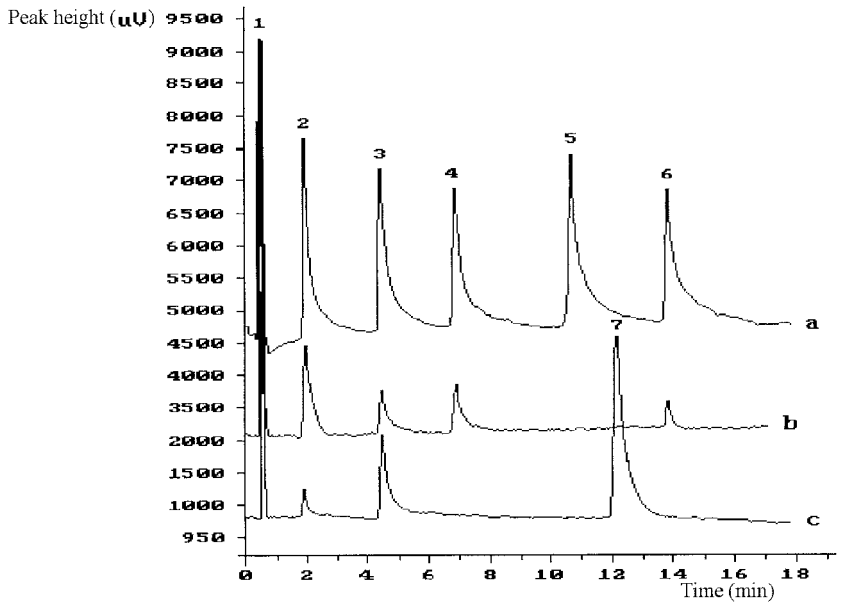

Figure 1: The GC-FPD chromatogram comparison of the standards, urine and blood. a: tin standards; b: urine; c: blood sample. Peaks identified as: 1. solvent (1μl cyclohexane); 2. TMT; 3. Internal standard; 4. DMT; 5. MMT; 6. Sn(IV); 7. Unidentified compound

Using internal standard method, the concentrations of all organotins in urine and blood samples were determined. The results were listed in Table 2. It showed that the urine samples contained several organotins and their contents were all at ng/ml level and in the blood sample, ng/g level of trimethyltin was detected and the peak

of the unidentified compound was rather high.

Table 2. The concentrations of DMT, TMT and Sn(IV) in each sample [a]

sample	DMT	TMT	Sn(IV)
Urine 1 ng/ml	79.7±1.8	83.3±2.3	66.4±3.1
Urine 2 ng/ml	20.4±0.6	42.0±1.0	47.2±0.8
Blood ng/g	ND [b]	70.0±3.3	ND

a. five times replicated measurements
b. not detected

According to the results shown above, it is clear that the patients were seriously poisoned and the concentrations of organtins in their bodies were rather high. Because the contaminated lards mainly contained dimethyltin compounds and the contents of trimethyltins in the urine and blood relatively increased compared with that in lards, we can infer that after dimethyltin compounds were absorbed and metabolized in vivo, some of them might be methylated to form trimethyltin compounds. It's well known that the most poisonous organotins investigated to date include trimethyltin derivatives. So trimethyltin compounds are drastic toxic and they can greatly harm human body. The action mechanisms of each tin compound on human body are still unknown to date.

The total amounts of 11 trace metals were determined by ICP-MS. The results are shown in Table 3. It's clear that in the two urine samples, tin is the majority metal

Table 3. The total amounts of trace metal elements (ng/ml)

element	urine 1	urine 2	blood
Cr^{52}	81.9	85.0	168.0
Co^{59}	1.3	ND	163.2
Ni^{60}	ND	ND	432.6
Cu^{65}	ND	ND	385.1
Zn^{66}	199.3	66.1	25.3
Ge^{74}	ND	1.6	458.8
Se^{82}	33.9	3.3	982.2
Cd^{112}	9.4	4.0	396.0
Cd^{114}	4.6	1.9	311.8
Sn^{118}	232.0	127.3	116.2
Sn^{120}	182.0	127.4	81.9
Hg^{202}	ND	5.04	903.8
Pb^{208}	17.8	13.8	259.5

ND = not detected

whose total amount is well above others, while in the blood sample, the contents of most metals are higher than that in urine samples and the concentration of tin is relatively low. This phenomenon can be explained by the fact that the three samples were from three different patients who might be poisoned by the contaminated lards in different containers. Because the chemical forms of these

trace metals except tin are unknown, their impacts on the patients are unable to be estimated. From the urine samples, it can be concluded that tin compounds were the main pollutant that made people ill, while from the blood sample, all metals except Zn whose concentration is relatively low may act. The amounts of total tin measured by ICP-MS well accorded with the data measured by GC-FPD, which indicated the validity of the two methods.

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