Speciation analysis of organotin compounds in lard poisoning accident in Jiangxi Province, China

JIANG Guibin (江桂斌), ZHOU Qunfang (周群芳), HE Bin (何 滨)
& LIU Jiyan (刘稷燕)

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
Correspondence should be addressed to Jiang Guibin (email: gbjiang@mail.rcees.ac.cn)

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Abstract Samples including organotin contaminated lard, urine, blood, and main organs of the poisoned bodies were collected in the incident which took place in Longnan and Dingnan counties, Jiangxi Province, China around the new year's day of 1999. The organotin compounds in these samples were identified and determined by gas chromatography-flame photometric detector (GC-FPD), gas chromatography-mass spectroscopy (GC-MS) and inductively coupled plasma-mass spectroscopy (ICP-MS). Experiments confirmed that tri- and dimethyltin are the main components that caused the poisoning accident. Monomethyltin, dioctyltin and inorganic tin were also found in several samples.

Keywords: organotin, pentylation, GC-FPD, GC-MS, ICP-MS.

The extensive use of butyltin compounds as antifouling paint has seriously polluted the harbor and estuary water, which has caused much attention in the world. But the environmental contamination resulting from industrial and agricultural usage of organotins has not been taken into serious account yet. Around the new year's day of 1999, in Longnan and Dingnan counties, Jiangxi Province, more than 1 000 people were seriously poisoned by the industrial lard contaminated with organotin. Over 100 people were hospitalized and three of them died. In this paper, gas chromatography coupled with flame photometric detector using quartz surface-induced luminescence (GC-FPD), gas chromatography-mass spectroscopy (GC-MS) and inductively coupled plasma-mass spectroscopy (ICP-MS) were used for analyzing the samples collected from Longnan and Dingnan counties. These samples included the contaminated lard, the urine and blood of the patients and the organs of the dead bodies. Experiments confirmed that di- and trimethyltin are the main components causing this accident. Monomethyltin, dioctyltin, inorganic tin and several trace metal elements were also detected in some samples as well. To our knowledge, this is the first case of organotin compounds especially methyltin species found in human organs.

1 Experimental

1.1 Reagents

Trimethyltin chloride (TMT, 98%), dimethyltin chloride (DMT, 97%) and monomethyltin (MMT, 97%) were obtained from Aldrich Chem. Co. (USA). Suitable amount of the compound

was directly weighed and dissolved in methanol to form a concentration of 1 mg/mL (as Sn), which was used as the stock solution and stored in refrigerator maintained at 4°C. Working standard solution (10 µg/mL) was prepared by diluting the stock solution with cyclohexane.

The Sn (IV) stock solution was obtained by dissolving metal Sn in 6 mol/L HCl solution, and then diluting to 4 mg (Sn)/mL with methanol. Working solution (5 µg/mL) was obtained by diluting the stock solution with cyclohexane-acetone (1 : 1).

Diocetyl tin oxide (DOT, pure) was bought from M&T Chemical Inc. (USA). Because DOT is difficult to dissolve in common organic solvents, the oxide was weighed and dissolved in 12 mol/L HCl in a 40°C water bath. When the white solid completely dissolved, the solution was diluted with methanol to form a concentration of 0.2 mg/mL (as Sn) as a stock solution. When it was diluted with cyclohexane-acetone (1 : 1), the standard working solution (5 µg/mL) was ready.

Grignard reagents of *n*-pentylmagnesium bromide (*n*-PeMgBr, 2.0 mol/L) and *n*-propylmagnesium bromide (*n*-PrMgBr, 2.0 mol/L) were prepared in the laboratory according to the standard synthetic methods^[1]. Monomethyl-tri(*n*-propyl)tin (MeSn (*n*-Pr)₃) used as internal standard (IS) was obtained by propylation of standard compound MeSnCl₃ (10 µg/mL, cyclohexane). All these preparations were confirmed by GC-MS.

1.2 Instrumentation

1.2.1 GC-FPD. A GC-9A gas chromatograph (Shimadzu, Japan) with a 25 m × 0.32 mm i.d. HP-1 capillary column coated with a film of 0.17 µm was used throughout the experiment. The separation was achieved by a temperature program of 50°C (hold for 2 min) to 200°C (hold for 5 min) at 10°C/min. Nitrogen (high purity) served as carrier gas and the column head pressure was 0.26 MPa. A laboratory-made flame photometric detector (FPD) using quartz surface-induced luminescence was used for monitoring organotin compounds, and the configuration and analytical figure of merits were described previously^[2,3]. The FPD was operated with a hydrogen-rich flame, hydrogen and air were controlled at 260 and 90 mL/min, respectively. The measurement was carried out by using a 394 nm interference filter. The temperatures of the injector and the detector were set at 220 and 160°C, respectively. Chromatograms were recorded on an SC-1100 PC data processing system.

1.2.2 GC-MS. A QP 5000 (HP, UK) GC-MS equipped with a fused silica capillary column (MDN-12, 30 m × 0.25 mm i.d. × 0.25 µm coating thickness) and a mass-selective detector was used for the identification of methyltin compounds. The GC temperature was programmed from 50°C (hold for 3 min), increasing by 30°C/min to a final temperature of 280°C with a hold time of 9.5 min. The injector temperature was controlled at 220°C. The carrier gas, nitrogen (high pure), was kept at an average pressure of 52.8 kPa on the top of the column and the total flow rate was 12.3 mL/min. The flow controller was split model with the split ratio controlled at 10 : 1. The

interface temperature was set at 250°C. The EI-MS detector's volt was 1.40 kV. Solvent cut time was 2.9 min. Acquisition time was 3—15 min. MS range was 40—400 (*m/z*) and the interval was 0.5 s.

1.2.3 ICP-MS. A Plasma-Quad 3 (VG Elemental, Winsford, UK) ICP-MS was used for the determination of total tin and other trace elements. General instrumental operating conditions were as follows: forward power, 1 350 W; reflected power, below 5 W; coolant argon flow rate, controlled at 14 L/min; auxiliary and nebulizer gas flow rate, controlled at 0.9 and 0.8 L/min, respectively; sample uptake rate, 1.0 mL/min; sample depth, 15 mm; nickel sampler orifice, 1.0 mm; nickel skimmer orifice, 0.7 mm; the first stage pressure, 1.6×10^5 mPa; the second stage pressure, 1.0×10 mPa; the third stage pressure, 1.7×10^{-1} mPa. Range-scanning mode was used for data acquisition. MS range was 50—210 (*m/z*) and the total acquisition time was 50 s.

1.3 Sample preparation

1.3.1 Sample preparation for GC-FPD and GC-MS. Owing to organotin ions' low volatilities and polar ionic properties, a derivatization step such as hydride generation with NaBH_4 ^[4], ethylation with NaBEt_4 ^[5], alkylation with Grignard reagent^[6], is needed before gas chromatographic separation. Here, pentylation by (n-Pe)MgBr was used for converting all tin compounds into their tetra-substitute ones, which are suitable for GC-FPD and GC-MS analyses.

For the preparation of pentylated standards, 0.10 mL of TMT (10 µg/mL), 0.15 mL of DMT (10 µg/mL), 0.20 mL of MMT (10 µg/mL), 1.0 mL of DOT (5 µg/mL), 0.50 mL of Sn (IV) (10 µg/mL) and 1.0 mL of internal standard MeSnPr_3 (2 µg/mL) were dissolved in 0.50 mL cyclohexane and reacted with 1.0 mL of 2.0 mol/L (n-Pe) MgBr for 15 min in an ultrasonic bath. The excessive Grignard reagent was destroyed by carefully dropwise adding 4 mL of 0.5 mol/L H_2SO_4 in ice water bath followed by an additional wash with 60 mL of deionized water. After manually shaken for 2 min, the solution was allowed to stand for 5 min for phase separation. The organic layer, containing the interested compounds, was separated, then dried and purified by anhydrous sodium sulfate (0.2 mg) and florisil (0.8 mg) which had been packed in a glass pipet (4.5 cm × 0.5 cm i.d.) and pre-washed with 5 mL of cyclohexane. The elution with cyclohexane was held on until the volume of the eluted solution was adjusted to 10 mL. Suitable volume of this sample was used for GC-FPD and GC-MS analyses.

For lard samples, suitable amount of lard samples was weighed and dissolved in 10 mL of cyclohexane. After 1.0 mL of internal standard MeSnPr_3 (2 µg/mL) was added, the rest procedures were similar to those of the standard preparation.

For urine and blood samples, 2 mL of urine sample (1, 2) or 0.40 g of the well-homogenized whole blood sample were placed in a separatory funnel together with 5 mL citric acid- NaH_2PO_4 buffer solution (pH = 5). 2 mL of MeSnPr_3 (80 ng/mL) were added as the internal standard. 2.5 mL of 0.1% tropolone-cyclohexane were added in sequence. The mixture was extracted for 15

min in an ultrasonic bath. After 10 min centrifugation at 2 000 r/min and removal of the supernatant, the residue was reextracted once again with another 2.5 mL portion of extraction solution. The combined organic phases were dried with anhydrous Na_2SO_4 and underwent pentylation, purification and dryness procedures just as the above description.

For organ samples, 0.4–0.8 g of wet organ after homogenization was placed in a 10 mL centrifuge tube fitted with a Teflon-lined cap. Mixed with 2 mL of the internal standard $\text{MeSn}(\text{n-Pr})_3$ (80 ng/mL), the sample was digested by 2 mL of $\text{KBr-H}_2\text{SO}_4$ solution (consists of 90 g of KBr , 27.5 mL of concentrated H_2SO_4 and 100 mL deionized water) and 0.5 mL of CuSO_4 (1 mol/L) for 15 min with vigorous shaking. The pH of the mixture was adjusted to 5 by adding 5.0 mL of citric acid- NaH_2PO_4 buffer. It was then extracted, derived, purified and dried as mentioned above. A suitable aliquot was injected into GC for separation.

1.3.2 Sample preparation for ICP-MS. An appropriate amount of samples including lard, urine, blood and organ were put into a 30 mL Teflon container, respectively and followed by addition of 2 mL concentrated HNO_3 . The container was then covered with a Teflon cap and heated on a hot plate kept at about 50°C for nearly 2 h, and then 0.5 mL of HNO_3 and 1 mL of concentrated perchloric acid (HClO_4) were added in sequence. The container was then put into a stainless steel bomb and sealed tightly which was heated again and kept at 170°C for 7 h. After the bomb being cooled down to room temperature, the container was opened and gently heated until the solution was evaporated into approximate 0.1 mL. The residue was dissolved in a small amount of 0.01 mol/L HNO_3 , and the solution was transferred into a 10 mL volumetric flask with the addition of 20 ng (1 mL) indium as internal standard and diluted to the mark with 0.01 mol/L HNO_3 to obtain the final solution used for ICP-MS analysis.

2 Results and discussion

2.1 Identification of organotin compounds in the samples

To date, many analytical methods facilitate the organotin speciation and many publications can be traced from literatures^[7]. Gas chromatography coupled with flame photometric detector using quartz surface-induced luminescence used here is widely used because of its high selectivity and sensitivity^[8]. All organotin and inorganic tin compounds in the lard, urine, blood and organs were separated and identified by GC-FPD after Grignard pentylation. Qualitative analysis was carried out by retention time comparison with standards. Methyltin compounds and inorganic tin existing in most of the samples were further confirmed by GC-MS.

2.1.1 GC-FPD analysis. The GC-FPD chromatograms of standard pentylated tin compounds and samples are shown in fig. 1. According to the retention time of the standards, each interested compound in the samples was identified. DMT was found in all of those lard samples and most of the lard contained MMT and TMT. One sample contained DOT and an unknown compound, and another contained Sn (IV). The patients' urine samples contained tri- and dimethyltin and inor-

ganic tin, and in the patients' blood, trimethyltin and another unknown compound were found. All organs of the died bodies contained tri- and dimethyltin and inorganic tin, while in the blank human organs (normal death case), only inorganic tin was detected.

Fig. 1. Comparison of the pentylated standard GC-FPD chromatogram with lard, urine, blood and organ samples. a, Pentylated standard tin compounds; b, the lard of Dingnan 3; c, the lard of oil shop; d, the blood of poisoned patient; e, the kidney of the poisoned decedent; f, the urine of the poisoned patient 2. 1, Solvent; 2, trimethyltin; 3, monomethyl-tripropyltin (internal standard); 4, dimethyltin; 5, monomethyltin; 6, inorganic tin; 7, dioctyltin; 8, unknown compound 1; 9, unknown compound 2.

2.1.2 GC-MS analysis. According to the analysis above, methyltin and inorganic tin compounds existing in samples were further confirmed by GC-MS. Full-scale monitoring with electron-impact ionization gives MS spectra of tin compounds. Fig. 2(a)–(d) show the fragmentation of MeSnPe_3 , Me_2SnPe_2 , Me_3SnPe and SnPe_4 , respectively. The spectra are characterized by clusters of isotope ion at each fragment. The isotope patterns created by ten tin abundant contributions are of particular use for recognition of any organotin compound occurring in a sample. The molecular ion $[\text{M}]^{+}$ is not observed for any of the pentylated tin compounds and the characteristic fragmentation pattern is dominated by successive cleavage of alkyl groups from $[\text{M}]^{+}$ with preferential cleavage of the largest alkyl group accompanied by the formation of $[\text{M}-\text{R}\pm 1]^{+}$ to $[\text{M}-\text{R}\pm 3]^{+}$ ions as usually observed. In fig.2 (a), monomethyltin is identified by the characteristic fragments of m/z 137 (SnMe^{+}), m/z 207 (SnMePe^{+}) and m/z 276 (SnMePe_2^{+}); in fig. 2 (b), m/z 135 (SnMe^{+}), m/z 151 (SnMe_2^{+}) and m/z 219 ($\text{SnMe}_2\text{Pe}^{+}$) are the special fragments of dimethyltin; in fig.2 (c), trimethyltin is characterized by the fragments of m/z 135 (SnMe^{+}), m/z 151 (SnMe_2^{+}), m/z 165 (SnMe_3^{+}) and m/z 221 ($\text{SnMe}_2\text{Pe}^{+}$); while the fragments of m/z 193 (SnPe^{+}),

m/z 264 (SnPe_2^+) and m/z 334 (SnPe_3^+) in fig.2 (d) ensure the existence of inorganic tin. Excellent matches are obtained in this study on the basis of the comparison between these mass spectra and the standard ones. A few of slight differences may come from the different instrument conditions or the different sample matrixes. So the results from GC-MS analysis further confirm the existence of methyltin compounds and inorganic tin in the samples.

Fig. 2. Mass spectra of pentylated methyltin and inorganic tin. (a) MeSnPe_3 ; (b) Me_2SnPe_2 ; (c) Me_3SnPe ; (d) SnPe_4 .

2.2 Quantitative analysis and comparison of the total organotin concentrations

Using internal standard method, the concentration of each organotin compound in the samples was measured by the determination of the peak heights of internal standard and the corresponding target compound. The results show that 8 lard samples contain several organotins and most of them are methyltin compounds. DMT is clearly the dominating pollutant with the mg/g concentration levels. DOT in lard of Longhua oil-shop is high too. While in urine and blood samples, the concentrations of trimethyltin and dimethyltin are almost equally high. Likewise, the same results are observed in the poisoned organs. It is well known that the toxicity of organotins increases with progressive introduction of organic groups at the tin atom, with maximum toxicity for trialkylated compounds and decreasing toxicity with increased length of organic moiety. The most poisonous organotins known to date are the triethyltin and trimethyltin derivatives. The acute

oral LD₅₀ value for trimethyltin acetate in the rat is 9 µg/g^[9]. The acute oral LD₅₀ values for many other organotin compounds in the rat are also at µg/g level. Because the average content of organotins in the contaminated lard is about 1 605 µg/g, if a man weighing 60 kg could consume 50 g oil a day in normal, the amount of organotin he ate reaches the same level of the LD₅₀ described above. Moreover, organotin compounds can be accumulated in the body. It is obvious that organotin compounds, mainly trimethyltin and dimethyltin, are the chief components which induce the ill effects on the human bodies and even result in death. The mechanisms of methyltin compounds in human bodies are not clear now. In addition, inorganic tin, which has been proved to be with little toxicity, is detected in almost equal amount in blank human organs (normal death case) and the poisoned organs. Thus, inorganic tin is not related to this accident. In order to understand the distribution of organotin compounds in food and human bodies, fig. 3 shows that the content of organotin compounds in lard is deadly high, while that in human bodies is no more than one thousandth. But that is enough to make great harmful effect to human bodies. Since the sources of urine, blood and organs samples come from different poisoned patients or decedents and their distributions in human bodies are different too, there are great disparities in organotin contents. The organotin content in the poisoned decedent organs is ten-folds more than that in poisoned patients, which obviously shows that the former is deadly poisoned. In conclusion, the analysis testifies that methyltin compounds in the contaminated lard are the material cause of the food poisoning accident.

Fig. 3. Organotin distributions in measured samples. The content of organotin in lard sample is 1.6 mg/g (3.21×500 mg/g).

2.3 Discussion of trace metal determination by ICP-MS

The total contents of 11 trace metals are determined by ICP-MS and the results are shown in table 1. It is clear that tin is the dominant pollutant in lard samples. It also widely distributes in urine, blood and organ samples where the content is relatively high. Consistency is obtained between the content of Sn detected by ICP-MS and GC-FPD, which ensures the reliability of the two methods. The content of Cd in a dead body's kidney and liver is also high. Because the detailed chemical forms of Cd in a dead body are unclear, their toxic effect to human bodies could not be exactly estimated. As the lard is mainly contaminated with tin species, especially for dimethyltin and trimethyltin, it is believed that tin element should be responsible for this terrible food poisoning accident other than other trace metals.

Table 1 Contents of total tin and other trace metals in lard, blood, urine and organ samples

Samples	C _T ⁵²	Co ⁵⁹	Ni ⁶⁰	Cu ⁶⁵	Zn ⁶⁶	Ge ⁷⁴	Se ⁸²	Cd ¹¹²	Sn ¹²⁰	Hg ²⁰²	Pb ²⁰⁸
Lard	Dingnan 1	245	4	ND	ND	ND	ND	23 833	321 045	ND	72
	Dingnan 2	1 036	ND	216	3 726	4	182	17 491	299 204	45	124
	Dingnan 3	5 985	1	ND	2 097	7	345	46 195	912 979	ND	161
	Dingnan 4	ND	ND	ND	ND	ND	ND	ND	1	ND	ND
	Longnan 1	6 394	19	ND	18 216	8	200	116 056	2 308 216	ND	1 023
Urine	Longnan 2	4 052	9	215	3 680	19	158	2 411	30 464	ND	528
	epidemic (pre- vention station)	18 298	6	3 565	12 508	47	151	136 482	2 601 773	ND	934
	cook-oil (shop)	13 594	82	3 792	8 162	68	108	97 340	1 700 761	ND	779
Blood	1	82	1	ND	199	ND	34	9	182	ND	18
	2	85	ND	ND	66	2	3	4	127	5	14
Poisoned organs	stomach	552	9	156	1 380	3	51	767	837	18	139
	kidney	680	20	202	2 799	8	438	61 723	3 503	55	210
	liver	936	123	212	10 155	7	520	16 559	4 970	52	389
	heart	1 013	28	487	3 251	8	41	532	1 852	58	57
	stomach	169	7	53	1 523	1	23	649	29	288	80
Normal organs	kidney	249	9	86	206	3	225	24 790	187	9	183
	liver	484	39	214	1 122	5	165	4 738	100	34	515
	heart	121	4	9	433	ND	30	88	41	5	54

ng/mL for blood and urine, ng/mg for lard. ND: no detection.

3 Conclusion

Experimental results, by qualitative identification and quantitative determination of all the samples collected from Jiangxi Province, show that the lard is contaminated with several kinds of organotin compounds, while dimethyltin is the dominant pollutant. In urine, blood and organ samples, the high contents of virulent trimethyltin and dimethyltin could directly disturb the body's metabolism, even cause death. This tragedy reminds us that we should pay much attention to this kind of poisonous compound and immediate action should be taken on the strict management of organotin.

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