APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2001; **15**: 933–938
DOI: 10.1002/aoc.246

Speciation and detection of organotins from PVC pipe by micro-liquid chromatography–electrospray–ion trap mass spectrometry†

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This paper describes the application of a micro-liquid chromatography–electrospray–ion trap mass spectrometry (μ-LC–ES–ITMS) method for separation and detection of organotin compounds leached from potable-water polyvinyl chloride (PVC) pipe. Dibutyltin (DBT) is added as a heat stabilizer to PVC. DBT was determined in 1 l water samples that had remained static in PVC pipes over several days (totaling 96 h). Other organotin compounds in the leachate were screened for, by using μ-LC–ES–ITMS. An initial level of approximately 1 $\mu g l^{-1}$ of DBT resulted within 24 h, with a subsequent drop and then a rise in DBT levels over the next 96 h to 0.8 $\mu g l^{-1}$. Published in 2001 by John Wiley & Sons, Ltd.

Keywords: organotin; potable water; PVC pipe; mass spectroscopy

Received 10 January 2001; accepted 30 May 2001

INTRODUCTION

Di-organotin compounds (e.g. dibutyl- and dimethyl-tin) have a large market as heat and UV-oxidation resistance stabilizers for rigid polyvinyl chloride (PVC) in industry. PVC products are widely used for potable-water pipe, cable, windows, siding, and roofing in the construction industry, and in bottles and packaging materials in the food, beverage, and household goods sectors.

It has been shown that organotin compounds can elicit a wide range of endocrine- and nervous-system effects, depending on the nature and number of alkyl groups bonded to the tin atom.[1] Organotin compounds show a wide variety of adverse health effects in many species, including imposex in mollusks, neural degeneration in fetal rat cell cultures, and induction of diabetes in hamsters.[2–4] Specifically, dibutyltin (DBT) has been shown to cause significant neurotoxicity in immature brain cell cultures at 30 ppb, to cause 100% suppression of natural killer lymphocytes function at approximately 3 ppm and 30% suppression at 150 ppb, and to be a potent teratogen if exposure is during the period of organogenesis.[5–7] Fent and Stegeman[8] indicate important biochemical effects of butyltins in fish, and they suggest that exposure to butyltins may alter both cytochrome P450-dependent metabolism and the induction response to other environmental pollutants.

The suspected origin of the organotin compounds in the marine aquatic environment is mostly from the use of marine antifouling paints that contain tributyltin(TBT), but it has been suggested that leaching and normal weathering of PVC products also contributes butyltins to the fresh water aquatic, and terrestrial, environments.[9] PVC pipe is widely used, throughout North America and Asia, as a domestic water supply carrier from main water lines into the home. Two recent studies by Sadiki and coworkers examined organotin compounds in Canadian drinking water that had been supplied by PVC pipe.[10,11] In those two studies, either dimethyltin (levels ranging from not detected to 291 $ng_{Sn}\ l^{-1}$), or DBT was detected (levels ranging from not detected to 52 $ng_{Sn}\ l^{-1}$). Other studies

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have examined the leaching of organotin compounds from PVC and chlorinated PVC (CPVC — a form of PVC used for high-temperature applications). All of these studies used methods that required either hydrolysis or derivatization of the organotin compounds before analysis by either gas chromatography (GC), GC–microwave-induced plasma atomic emission spectrometry, atomic emission detection, or atomic adsorption spectrometry detection.[9–14] These methods, though sensitive, are not definitive. The use of complexing agents can produce high background interference, and lead to incomplete hydrolysis and derivatization. Other researchers have used liquid chromatography–ionspray mass spectrometry to characterize alkyl- and aryl-tin species and to quantify TBT in sediments.[15–17] Previous work from our laboratory has focused on method development for solid-phase extraction and micro-liquid chromatography–electrospray–ion trap mass spectrometry (μ-LC–ES–ITMS) detection of organotin compounds in water.[18] These LC–MS methods offer the advantage of fewer analytical steps, less reagents used, definitive identification of organotin species, and reduction of analysis time. The purpose of the research reported here was to apply a μ-LC–ES–ITMS method to speciate and quantify which organotins (if any) were leaching from PVC pipe into a static water matrix.

EXPERIMENTAL

Preparation of materials

Organotin standards, 96% TBT chloride, 96% DBT dichloride, and 95% monobutyltin (MBT) trichloride, were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Stock solutions (approximately 500 ng μl^{-1}) were prepared in high-performance liquid chromatography (HPLC)-grade methanol (Burdick & Jackson, Inc., Muskegon, MI, USA) and stored in darkness at 4 °C. The solvents used for both the extractions and the μ-HPLC mobile phase were HPLC-grade methanol (Burdick & Jackson, Inc., Muskegon, MI, USA), glacial acetic acid (J.T. Baker, Phillipsburg, NJ), deionized water (NANOpure™, Barnstead–Thermolyne, Dubuque, Iowa, USA), and tropolone (Aldrich Chemical Co., Milwaukee, WI, USA).

One inch diameter PVC pipe (schedule 40, potable-water approved) was obtained from Ace Hardware (Las Vegas, NV). Two different manufactures of PVC pipe were used in these studies: PW pipe (Brand 1) (Pacific Western Extruded Plastics Co., Eugene, OR, USA) and Genova PK II (Brand 2) (D. A. Fehr, Inc., Friedensburg, PA, USA). The PVC pipe-glue and solvent, approved for potable-water use, was obtained from Ace Hardware (Las Vegas, NV); the Ace PVC cement and Ace purple primer were manufactured by Ace Hardware Corp. (Oakbrook, IL, USA).

Note: mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

Leaching study I

Four sets of PVC pipe (Brand 1), labeled A, B, C, and D, were measured and cut to hold 1 l of water. Pipe A consisted of two 40″ (2 × 1.02 m) sections glued together, for a total of 80″. Pipes B, C, and D were each cut as one continuous length of pipe, 80″ long (2.03 m).

On one end of each pipe a PVC cap was permanently affixed with PVC glue (approved for use with potable water), then 1 l of deionized water (average pH 5) was placed into each pipe, A–D. After filling the pipe the other end was capped with a removable PVC cap (so that it could easily be removed in order to fill and empty the waters out). The waters in pipes C and D were spiked to contain 25 $\mu g\ l^{-1}$ of DBT (using the same stock standard solution) each time they were re-filled for the specified holding periods. All of the waters (A–D) were held static, in a vertical position, at room temperature for specific periods of time: 24, 48, 72, and 96 h. At the end of each time period (e.g. 24, 48, 72 or 96 h) the 1 l of water was removed for extraction and analysis by μ-LC–ES–ITMS, and another 1 l of water was poured into the pipes for the next time period. The pipes were not rinsed between each static holding period.

Leaching study II

Four sets of PVC pipe (Brand 2), labeled A, B, C, and D, were measured and cut to hold 1 l liter of water. Pipe B consisted of two 40″ (2 × 1.02 m) sections glued together, for a total of 80″. Pipes A, C, and D were each cut as one continuous length of pipe, 80″ long (2.03 m). Leaching study II then followed the same procedures as stated previously for study I.

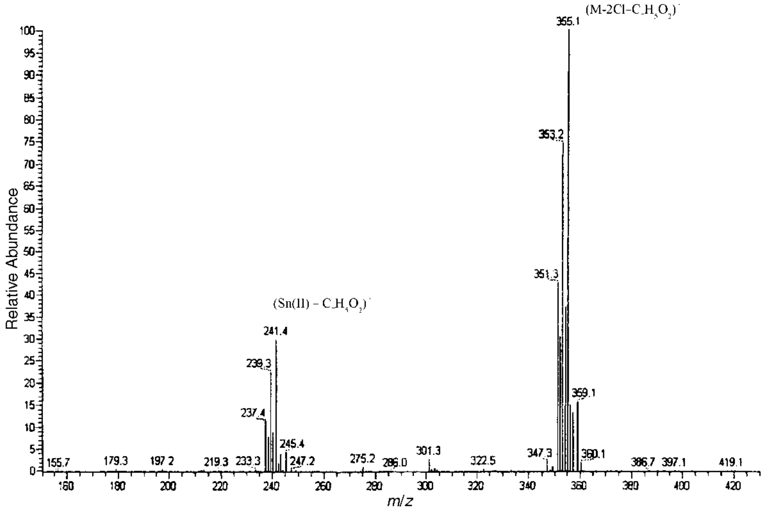

Figure 1 Mass spectrum of DBT dichloride.

Extractions

After the appropriate amount of time (e.g. 24, 48, 72, and 96 h), the water samples were removed and poured into the extraction apparatus. Extractions were performed with a CPI International (Santa Rosa, CA) Accuprep 7000™ manifold and glass reservoirs containing either CPI Nu-Phase™ fiber C_{18} solid-phase extraction (SPE) disks (Santa Rosa, CA), or 3M Empore™ C_{18} SPE disks (3M Company, Roseville, MN, USA). Previous in-house studies conducted by the authors have seen little or no difference in the extraction efficiencies of the organotins between the two C_{18} disc manufacturers. The disks were prepared for extraction according to manufacturers' instructions. The water samples were adjusted to approximately pH 2.5 with HCl (12 M), then allowed to pass through the prepared SPE disks under gravity flow (after initiating flow *via* vacuum) for 30 to 40 min. The organotin compounds were eluted from the disks with three 10 ml volumes of 99% methanol–1% acetic acid, for a total of 30 ml. The organic extracts were evaporated to 0.5 ml using a TurboVap® II (Zymark Corporation, Hopkinton, MA, USA), with nitrogen gas.

μ-LC–ES–ITMS analysis

The extracts were analyzed for organotin compounds by μ-LC–ES–ITMS, the details of which are described elsewhere.[18] The micro-capillary columns were prepared in-house, as described by Moseley *et al.*[19] An isocratic mobile phase of 80% methanol, 14% water, 6% acetic acid, and 0.1% tropolone (v:v:v:m) was used at flow rates of 5 to 6 μl min^{-1}.[20] The analyses were done using a Finnigan LCQ™ (ThermoQuest, San Jose, CA, USA), configured with an ES ion source, with the analyzer set in the positive ion mode. The LCQ™ is an ion trap mass spectrometer detector that performs real-time mass analyses of liquid chromatograph eluents over a mass-to-charge ratio range of 50 to 2000. The ES needle was run at approximately 4.5 to 5.2 kV, and the ion trap mass spectrometer was scanned from 150 to 430 amu (full-scan mode) in three μ-scans with an ion injection of 200 ms.

RESULTS AND DISCUSSION

The use of μ-LC–ES–ITMS allowed for the unequivocal determination of which species of organotin was leaching from the PVC pipes. The mass spectra of the organotins are very characteristic, in that tin has ten isotopes, thereby creating a clear and distinct mass spectral pattern. The definitiveness of DBT being present in the water leachates was based upon identifying the spectrum of DBT in the extracts. The spectrum, shown in Fig. 1, of DBT dichloride (MW = 304 Da) has only two ions present attributable to DBT. The base ion, *m/z* 355, is experimentally attributable, by ES–ITMS, to the DBT tropolonium ion, $(Sn(C_4H_8)_2C_7H_5O_2)^+$.

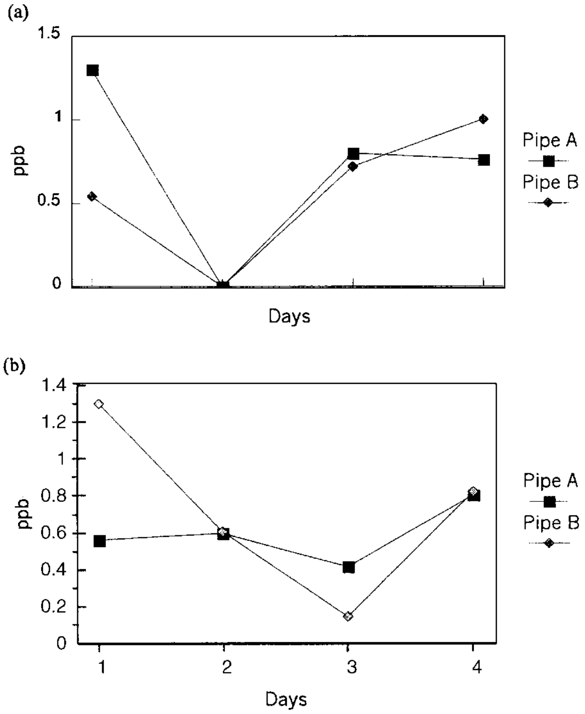

Figure 2 (a) Leaching study I — DBT; (b) leaching study II — DBT.

Another ion (*m/z* 241), present in the spectrum at 15% relative abundance, can result from the reduction of tin(IV) to tin(II) plus one tropolonium ion, $(\mathrm{Sn(II)} + C_7H_5O_2)^+$. The presence of tin(II) ions is not unexpected and has been seen by one of the authors in previous work, and other researchers have shown that charge reduction of metal ions can occur in the electrospray source.[21,22] The other butyltins, TBT and MBT, were also screened for. TBT was not present above the limit of detection (LOD) of this method (LOD = 780 pg).[18,23] However, there was spectral evidence of MBT (*m/z* 419, $\mathrm{Sn}(C_4H_8)(C_7H_5O_2)_2]^+$) present in some of the spectra obtained from the sample extracts, but all the detections were less than the limit of quantification (LOQ) of 3 ng.[23]

In study I (Brand 1) the level of DBT leaching from pipe B (a whole section of pipe) starts out at about 0.6 ppb, drops below the LOD at 48 h (for DBT the LOD = 970 pg), and then slowly rises to about 0.8 ppb at 96 h.[18,23] Similar results were seen in study II (Brand 2). In pipe A (whole section of pipe) the initial level of DBT was 0.6 ppb, remained the same over 48 h and 72 h, and then rose to 0.8 ppb at 96 h (Fig. 2).

Table 1 Leaching study I (Brand 1)

Hold time	DBT in leachate ppb (% RSD)[a]		DBT spike % recovery	
(h)	Pipe A[b]	Pipe B	Pipe C	Pipe D
24	1.3 (9)	0.58 (8)	102	41
48	ND[c]	ND[c]	47	39
72	0.56 (14)	0.80 (15)	24	32
96	0.76 (17)	0.82 (9)	65	63

[a] % RSD is % relative standard deviation of triplicate injections from same sample extract.
[b] Pipe A in study I was two pieces of 40″ pipe glued together.
[c] ND = not detected–below detection limits.

What was surprising was that there were initial differences in leaching of DBT from the pipes that were cut and glued together [pipe A, study I (Brand 1), and pipe B, study II (Brand 2)] versus the pipes that were whole sections [pipe B, study I (Brand 1), and pipe A, study II (Brand 2)]. Tables 1 and 2 show that the amounts of DBT that leach from the cut pipes were similar. The initial levels of DBT leaching from the cut-and-glued PVC were around 1 $\mu g\ l^{-1}$ in both studies; in comparison, the initial levels of DBT leaching from the whole PVC, in studies I and II, were 0.58 $\mu g\ l^{-1}$ and 0.56 $\mu g\ l^{-1}$ respectively. There was an increase of almost two fold between pipe that had been cut and glued back together and uncut pipe. Without further studies, it is unknown whether the higher level, initially seen in the cut pipes, is due to the interaction of the primer and glue on the pipe and subsequent solvation of DBT from the pipe, or whether DBT is present in the glue and primer and is dissolving into the water.

In comparing the results of this study with those of others, we find similar results. Wu *et al.*,[14] for example, where water flowed continually (0.35

Table 2 Leaching study II (Brand 2)

Hold time	DBT in leachate ppb (% RSD)		DBT spike % recovery	
(h)	Pipe A	Pipe B[a]	Pipe C	Pipe D
24	0.56 (14)	0.96 (0.6)	71	17
48	0.60 (12)	0.61 (5.4)	34	40
72	0.57 (6)	0.35 (11)	37	47
96	0.80[b]	0.82[b]	33	39

[a] Pipe B in study II was two pieces of 40″ pipe glued together.
[b] Only single injections were made on these samples.

$m^3\,h^{-1}$) through schedule 40 PVC pipe during a 4 day period, found that the total tin leaching from PVC ranged from 1 to 5 $mg\,m^{-3}$ (where 1 $mg\,m^{-3}$ is equivalent to 1 $\mu g\,l^{-1}$ standard for tin).[14] In our study using a static mode, a maximum of 0.8 $\mu g\,l^{-1}$ of DBT was found to leach from the PVC after 4 days (i.e. 96 h). In other studies, where PVC chips were used instead of whole pipe, a maximum of 200 $ng\,l^{-1}$ of total tin was reported after 30 days.[9] Another study examined organotin leachates from CPVC pipe.[13] CPVC is used in homes for hot-water applications. The CPVC study found levels up to 41.7 $ng_{Sn}\,ml^{-1}$ (reported as DBT); this higher level (higher than that reported in our paper at 0.8 $ng_{DBT}\,ml^{-1}$) may have resulted from the elevated temperature (65 °C) of the water.[13] The CPVC study did find lower levels at a lower temperature of 24 °C: 2.4 $ng_{Sn}\,ml^{-1}$ (reported as DBT), comparable to the 0.8 $ng_{DBT}\,ml^{-1}$ reported in this paper.[13]

In both studies I and II, each time a new holding period was started, duplicate sets of pipes were each filled with 1 l of water and spiked with 25 $\mu g\,l^{-1}$ of DBT. These 'spiked' pipes were held for the same amount of time as the experimental pipes, and the waters were removed and extracted like the others in the studies. This was done with the idea of having a quality assurance measure to see how well the extraction efficiency of this method, as applied to the leachate from the pipes, would be. However, there were many inconsistencies in the results from the spikes. The overall recoveries were very variable, ranging from 17 to 102%.

CONCLUSIONS

The DBT values reported in Tables 1 and 2, were obtained from triplicate injections of each sample extract, the %RSD values ranged from <1 to 17% with the µ-LC–ES–ITMS method. In comparing the results from this paper with those produced by others, it can be seen that although there is some variation in the methods of analysis of the various leaching studies, the results are similar. Though some of this variability could be due to the differences in methodologies, the overall conclusion that can be drawn is that DBT can leach from PVC pipe (schedule 40) into water, under normal, ambient conditions, and in concentrations averaging 1 $\mu g\,l^{-1}$ (averaged from the different studies, including this one). This reported value is within an order of magnitude for neurotoxicity, as reported in Ref. 5 (in that study, both immature and differentiated cultures of neural cells exhibited signs of cell type-specific toxicity when exposed, *in vitro*, to concentrations of 30 $\mu g\,l^{-1}$ of DBT).[5] There is also evidence, from this study, that cutting and glueing PVC pipe can increase the initial levels of DBT leaching out of the pipe. This increase is perhaps due to the increase in exposure of the interior surface of the pipe, and the solvation of the glue and primer on the pipe, thereby releasing DBT; or, perhaps, the DBT is coming from the primer and glue itself.

The inconsistencies of the results from the spiked waters show that several factors need to be considered involving the leaching and degradation processes of organotins: (a) adsorption processes between the PVC surface and the analytes; (b) possible degradation of DBT to MBT (although MBT was detected in some of the non-spiked extracts it was below our LOQs during this study) and inorganic tin species; and (c) DBT has been shown to be more adsorptive than MBT or TBT in storage experiments.[13] In reviewing the spiked data, it probably would have been a more appropriate measure of extraction efficiency if the waters spiked with DBT had been held for the same time and conditions, minus the wall interaction, in a non-sorbing container.

Acknowledgements The US Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded and performed the research described. This manuscript has been subjected to the EPA's peer and administrative review and has been approved for publication.

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