They're everywhere ... ORGANOTINS ... Are they coming to you?

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1 Introduction

Everyone should be concerned with the issue of hormonal disruptors aka endocrine disruptors. A recent health perspective article in USA Weekend, Feb. 13-15, 1998 entitled, "Are common chemicals scrambling your hormones?," brings the issue of hormonal disruptors to the public eye.

There are many studies that are currently available on the effects of organotins on all living organisms. Organotin compounds elicit various toxicological properties, including endocrine disruptors, depending on the nature and number of alkyl groups bonded to the tin atom.[1] Butyl- and phenyltin compounds, particularly trisubstituted species, are known to be very toxic to marine organisms at very low concentrations (sub-ppb).[2] Researchers have documented a range of effects of organotins:

- cause *imposex* (the imposition of male characteristics upon the female) in molluscs
- cause the inhibition of cytochrome P4501A in fish
- cause hematological and pathomorphological alterations in rainbow trout
- cause diabetogenic effects in hamsters
- cause neural degeneration in fetal rat cell cultures
- cause deaths of dolphins by suppressing their immune systems
- can be a teratogen to mammals during organogenesis (gestation)

2 Where Are They Coming From?

- Plastics . . . . . Dibutyltin is used in many plastic products.
- Agriculture . . . . . Triphenyltin is used on crops, e.g. sugar beets, rice, and pecans.
- Antifouling paints. . . . Tributyltin is widely used on ships.

The suspected origin of the organotins in the aquatic environment is mostly from the use of marine antifouling paints (containing tributyltin). It has also been shown that leaching and normal weathering of PVC products and plastics contributes dibutyltin, and its decomposition products, to the aquatic and terrestrial ecosystems.[3]

Organotins used as disinfectants, stabilizers for plastic polymers and as catalysts for epoxyresins are primary demyelination routes for the brain.[4] Organotin compounds are a class of oligodendrolglial toxins which affect the central nervous system (CNS) which causes primary demyelination of the brain. Some classes of organotins can affect the peripheral nervous system (PNS) as well.

3 Methodology

EPA's current method for analyzing tin is for total tin, not the speciation of tin. Speciation of tin is important due to the toxicological of effects the various congeners of tin. Rather limited use has been made of mass spectrometry in the study of organotin compounds though ms linked to gas-liquid chromatography is now being used for the identification of organotins, particularly in environmental studies.[5] This relatively simple and quick extraction method achieves low detection limits of organotins in the low nanogram to high picogram range. The separatory-determinative method utilizes μ-liquid chromatography-electrospray/ion trap mass spectrometry (μ-LC-ES/ITMS). Low solvent use and no hydrolysis or derivation makes this μ-LC-ES/ITMS method quick and easy.

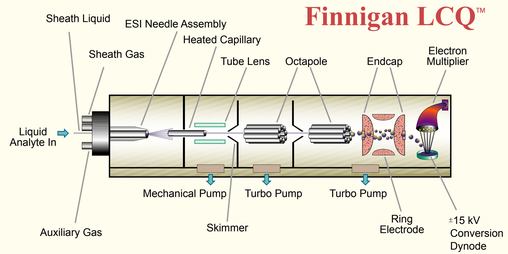
Chromatography

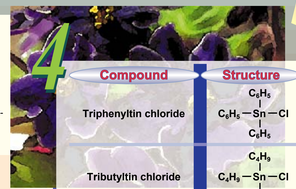
Micro-capillary columns were prepared in-house with 160 μm i.d. (360 μm o.d.) fused silica columns were packed with approximately 10 to 12 cm of 5 μm ODS-Hypersil.

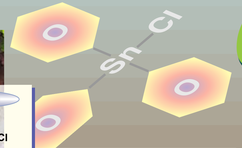
Mass Spectrometry

A Finnigan LCQ™, configured with an electrospray (ES) ion source, was used to detect the organotins. The LCQ™ is an ion trap mass spectrometer (ITMS) detector that performs real-time mass analyses of liquid chromatography (LC) eluent over a mass-to-charge ratio of 50 to 2000. The ES needle was run at approximately 4.5 kV to 5.2 kV, and the ITMS was scanned from 150 to 430 (full scan mode) in 3 μscans with an ion injection of 200 ms.

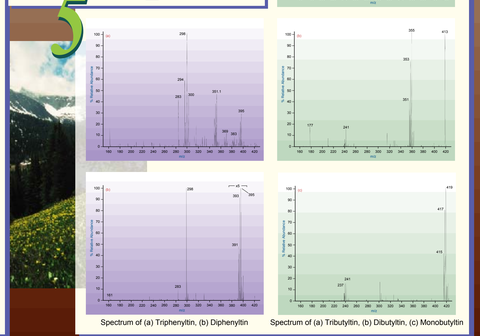
Finnigan LCQ™







Compound	Structure
Triphenyltin chloride	C_6H_5 — Sn — Cl (C_6H_5, C_6H_5)
Tributyltin chloride	C_4H_9 — Sn — Cl (C_4H_9, C_4H_9)
Dibutyltin dichloride	C_4H_9 — Sn — Cl (C_4H_9, C_4H_9, Cl)



Spectrum of (a) Triphenyltin, (b) Diphenyltin

Spectrum of (a) Tributyltin, (b) Dibutyltin, (c) Monobutyltin

6 Sample Preparation

Brain Tissue Study

The procedure for extracting organotin compounds from tissues consisted of a liquid-liquid extraction. The extraction was carried out by sonication and centrifugation. The samples were placed in racks and the solvent solution, a mixture of hexane, acetic acid, and tropolone (99:1:.1v/v) serially added. The samples were then sonicated for 15 minutes in an ultrasonic bath. After sonification the samples were centrifuged for a period of 15 minutes at 4000 rpm. After collection, the sample is evaporated down to 0.5mL by using a moderate flow of nitrogen in the TURBOVAP.

Natural Water Study and PVC Pipe Studies

Time course exposures of 24, 48, 72 and 98 hours were used to determine the leaching of organotins from PVC pipes. Various river water samples were collected. Extractions were completed using a manifold in combination with C_{18} solid-phase extraction (SPE) disks. All water samples were pH adjusted to 2.5 pH with HCl then gravity extracted. The organotins were eluted serially from the disks with a solvent solution of methanol and acetic acid (99:1v/v). The extractant were evaporated to 0.5 mL with the TURBOVAP.

7 Results

Water Study

A new technique combining SPE discs for extraction and μ-LC-ES/ITMS for separation and detection was developed to detect organotins in water. The limit-of-detection (LOD) with this method is 750 pg, on-column, using three times the signal-to-noise method. Deionized water was spiked between 7 to 10 ppb for dibutyltin, tri- and di-phenyltin, while tributyltin was spiked at 20 ppb. The average recovery of tributyltin was 86% and dibutyltin was 82%, with relative standard deviations (%RSDs) of 43% and 37%, respectively. The average recovery of triphenyltin and diphenyltin from water was 78% and 56%, respectively, with %RSDs of 21% and 36%.

PVC Study

Duplicate time course exposures of 24, 48, and 72 hours were used to determine the leaching of organotins from PVC pipes. Both pipes were cut to hold exactly 1-L of water, from 1" PVC pipe. The results from study I and II are shown in the "PVC leaching" graph.

It is interesting to note that in both studies one of the pipes (in each study) was composed of two equal lengths of PVC glued together, with PVC potable water approved glue, and showed a higher starting level than that composed of the single length of pipe.

Tissue Study

Aggregate brain cell cultures were used to test the neurotoxicological potential of dibutyltin (DBT) with a 10-day treatment and concentrations ranging from 10^{-10} M to 10^{-6} M, either during an early developmental period, or during a phase of advanced maturation. DBT caused significant cell loss at 10^{-7} M in immature cultures, and at 10^{-6} M in differentiated ones. DBT treatment in immature cultures affected predominantly the glial parameters and the cholinergic neurons, whereas in mature cultures, all cell types decreased with the same pattern. While TMT is known to be neurotoxic and induce gliosis, and TET to cause demyelination, DBT appeared to be more toxic than TMT and to present a distinct development-dependent myelinic toxicity compared to TET. These differences among organotin compounds in their effects on brain cells suggest different pathways of action. This work was conducted at the Lausanne Institute de Physiologie, by Dr. Florianne Monnet-Tschudi.

Our research at ECB was to use μ-LC-ES/ITMS to identify, confirm and quantitate the DBT that was spiked into the culture flasks. With only a couple of exceptions, at the 10^{-7} M level, the results of the mass spectrometry quantitated amounts were close to those amounts spiked into the culture flasks. One anomaly noted was that there was some DBT found in some of the control aliquot tubes. This is possibly due to the fact that the aliquot tubes, which were made of a plastic-type material, contained some amount of DBT. It is possible that the extraction method employed extracted the DBT out of the walls of the aliquot tubes.

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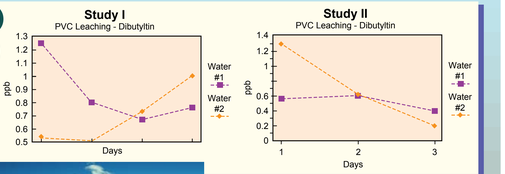

9 Conclusions

A new technology had been developed utilizing μ-LC-ES/ITMS to separate, speciate, and detection of organotins. This has resulted in a low-cost, easy to use method which requires no hydrolysis or derivatization. This method allows very low levels of detection with a variety of matrices.

10 References

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