

Stability studies of endocrine disrupting tributyltin and triphenyltin compounds in an artificial sea water model

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Abstract. Triorganotins belong to toxic components present predominantly in antifouling paints for marine vessels. Tributyltin/triphenyltin at pico- or nanomolar concentrations in sea water are known to induce an irreversible sexual abnormality in females of over 190 marine species, an “imposex” phenomenon – the superimposition of male genitalia on a female. Moreover, trialkyltins and triaryltins function as potent nuclear retinoid X receptors (RXR) agonists. In mammals, triorganotin compounds induce immunosuppressive, metabolic, reproductive or developmental effects. Toxic effects of triorganotins warrant the need for monitoring of their long-lasting presence in the environment. This study brings novel data on the stability of two triorganotin compounds in artificial sea water model obtained by applying ultra-pressure liquid chromatography (UPLC) and gas chromatography-mass spectrometry (GC-MS) methods. Stability of tributyltin and triphenyltin chlorides was studied for 180 days and the degradation kinetic parameters were obtained. Tributyltin chloride was the less stable with the degradation kinetic parameters $K_{deg} = 0.00014 \text{ day}^{-1}$ and $t_{1/2} = 4950 \text{ days}$ (13.6 years). K_{deg} of the more stable triphenyltin chloride was determined to be $K_{deg} = 0.00006 \text{ day}^{-1}$ with $t_{1/2} = 11550 \text{ days}$ (31.6 years). Since similar stability data of triorganotin compounds were not published previously, we report high stability for both tested compounds, which indicates a significant environmental problem when these substances enter sea water and later coastal sediments.

Key words: Triorganotins — Sea water stability — UPLC — GC-MS

Abbreviations: ER, estrogen receptor; FXR, farnesoid X receptor; GC, gas chromatography; LXR, liver X receptor; MS, mass spectrometry; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAR, all-*trans* retinoic acid receptor; RXR, retinoid X receptor; TR, thyroid hormone receptor; UPLC, ultra-pressure liquid chromatography; VDR, dihydroxyvitamin D₃ receptor.

Introduction

Triorganotin compounds are known as components of anti-fouling paints for marine vessels. They are also components of heat stabilizers in vinyl chloride polymers (PVC), wood preservatives, agricultural pesticides, disinfecting agents in industrial cooling waters and industrial catalysts in a variety

of chemical reactions (Kotake 2012). In general, organotin compounds are components of a variety of plastics, including silicone. Their occurrence is almost everywhere, e.g. in food containers, medical devices, toys, wallpaper, household piping, etc. (Sousa et al. 2014). Due to organotin compounds widespread use, organotins can be detected in soils and sediments, water and also in the atmosphere (Kannan et al. 2010). Triorganotin compounds, predominantly of tributyltin (TBT) origin have been largely released into water from antifouling paints, resulting in bizarre effects on aquatic organisms (Sousa et al. 2014). TBT at pico- or nanomolar concentrations was found to induce an irrevers-

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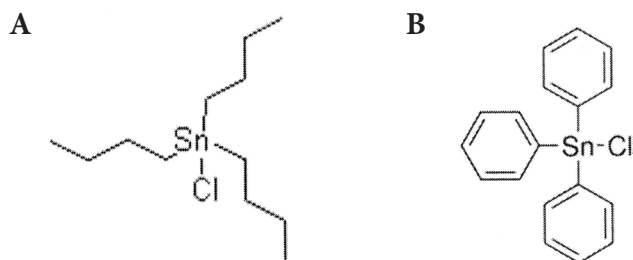


Figure 1. Chemical formulas of tributyltin chloride (A) and triphenyltin chloride (B).

ible sexual abnormality in female molluscs, a phenomenon known as “imposex” (the superimposition of male genitalia on female). In the other words, TBT can cause irreversible sex-organ alterations as a result of masculinization process by which a penis and a vas deferens develop in more than 190 marine species (Nakanishi 2008). Trialkyltins, e.g. tributyltin, and triaryltins, e.g. triphenyltin (Fig. 1), function as potent nuclear retinoid X receptors (RXR) agonists due to their capability to bind to the ligand-binding domain of RXR subtypes and function as transcriptional activators (Nakanishi et al. 2005, Brtko and Dvorak 2015). This phenomenon was very recently confirmed also by radioligand binding assay (Toporova et al. 2016). The effect of selected triorganotins on nuclear retinoid and retinoid X receptor subtypes expression in human breast cancer cells clearly confirmed their potency to affect nuclear receptors at the mRNA or protein level (Macejova et al. 2017). Since RXRs can act predominantly as heterodimeric partners of a number of other nuclear receptors, including retinoic acid receptors (RARs), thyroid hormone receptors (TRs), dihydroxyvitamin D₃ receptor (VDR), peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), farnesoid X receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), and several orphan receptors, then RXR subtypes play a crucial role in the modulation of many hormonal signals and regulatory pathways within the cells (Brtko and Dvorak 2011, 2015; Flodrova et al. 2016; Macejova et al. 2016). In mammals, organotin compounds also induce immunosuppressive, metabolic, reproductive or developmental effects (leMaire et al. 2009, Macejova et al. 2016).

All the interesting biological activities and toxic effect of triorganotin substances draw the attention to monitoring and regulation of their presence in the environment. Consequently, the development of modern sensitive and selective instrumental analytical techniques for the extraction, pre-concentration and determination these substances followed (Cole et al. 2015). This resulted in some data available on the presence of organotin substances in the

environment (Arp et al. 2014; Rodriguez-Cea et al. 2015; Suzdalev et al. 2015). However, no data are available on the rate of degradation of individual triorganotin substances in the sea environment.

The aim of this study was to bring novel data on the stability of tributyltin and triphenyltin chlorides in artificial sea water, which was achieved by using the ultra-pressure liquid chromatography (UPLC) and gas chromatography-mass spectrometry (GC-MS) methods.

Material and Methods

Preparation of solutions

On average, sea water in the world's oceans has a salinity of about 3.5% (35 g/l) of predominantly sodium and chloride ions. Stock solutions were prepared by dissolving 20 mg of each compound (tributyltin chloride or triphenyltin chloride) in 5 ml of 50:50 artificial sea water: methanol solution. Both triorganotin compounds were purchased from Sigma-Aldrich (Germany) with purity 96% and 95%, respectively, as declared by the chemical distributor. The solutions were sonicated for 30 min to ensure complete dissolution of the compounds. Working solutions were prepared by diluting 200 μ l of stock solution in 1 ml of mobile phase (80:20 v/v acetonitrile:H₂O).

UPLC instrumental conditions

High purity water was obtained using Milli-Q water purification system. HPL grade methanol and acetonitrile were used. The mobile phase was filtered through a 0.2 μ m nylon membrane filter from Millipore and degassed daily before use. Separation was achieved using a mobile phase that consists of 80:20 (acetonitrile:H₂O) at a flow rate of 0.4 ml/min in only 4-min runtime. The column temperature was maintained at 40°C, injection volume was 10 μ l and detection wavelength was set at 254 nm for determination of triorganotin chlorides.

The fast liquid chromatography was performed using UPLC system (Waters' Equipment Company, Inc., U.S.A.) with Acquity TUV detector. Chromatogram and data were recorded by means of Empower software. The chromatographic system was performed using Acquity BEH C18 (100 \times 2.1 mm) id, 1.7 μ m column.

Stability study

All solutions used in the study were prepared at an initial concentration of 4 mg/ml and put in an incubator (GFL, U.S.A) at 60°C for 1, 2, and 3 hours. All solutions were then diluted in the mobile phase to give a final concentration of

0.8 mg/ml and filtered before injection. Readings were taken at zero time, after 1, 2 and 3-hours incubation at 60°C. Readings also were taken after 3 days, 7 days and after 6 months of keeping solutions at room temperature.

GC-MS instrumental conditions

The GC-MS chromatography experiments were performed using Trace GC Ultra-ISQ (Manufacturer: Thermo Scientific, USA). All operating and data acquisition were processed by Excalibur software. Library search was done using the library NIST MS Search 2.0 of equipment. GC separation of analytes was achieved by TG-SQC GC column: 15 m × 0.25 mm × 0.25 μm (Thermo Scientific) using the following temperature program: Initial temperature of the experiment was 50°C with the hold time of 1 min and then 280°C with the hold time of 10 min at the rate 30°C/min. Total run time was 19 minutes.

Solutions of tributyltin chloride, triphenyltin chloride and tetraphenyltin chloride were prepared in methanol at concentrations 100 μl/ml and 100 μg/ml, respectively (according to the sample). An aliquot of 1–2 μl of each solution was injected into GC. The MS profiles were matched with NIST library of the instrument for detection.

The conditions used for MS analysis by ISQ Mass Spectrometer were: MS transfer line temperature of 250°C, ion source temperature of 200°C, EI (electrospray ionization) mode and mass detection (m/z ; mass/charge number of the ions) in the range of 50–450. Helium gas Grade 6 (99.9999%) at a flow rate of 1.2 ml/min was used.

Procedure

Solutions of tributyltin and triphenyltin chlorides were prepared in methanol at concentrations 100 μl/ml and 100 μg/ml, respectively. An aliquot of 1–2 μl of each solution was injected into GC. The MS profiles were matched with NIST library of the instrument for detection.

Kinetics calculation for organotins degradation

Based on the UPLC determination of stability after 6 storage of the solutions at the room temperature, the degradation kinetic parameters, K_{deg} and $t_{1/2}$, in artificial sea water were calculated (Carstensen 1995).

Results

GC-MS

The GC-MS analysis of the first compound – tributyltin chloride (TBT) showed GC peak at retention time (Rt) 6.04

min (Fig. 2A) with ion masses at m/z 292, 269, 213, 155 and 119 (Fig. 2B). These ions originated from the fragmentation of the parent compound – tributyltin chloride. The molecular peak of the parent molecule was not detected due to the low stability of the molecule under MS conditions. This is in an agreement with the data from the Excalibur library where the tributyltin chloride spectrum also does not contain the molecular peak of MW 326. As stated by the producer (Sigma-Aldrich, U.S.A.), GC showed the presence of some impurities of these compounds. This is in good agreement with the declared purity of used compounds, which declared purity of tributyltin chloride and triphenyltin chloride equal to 96%. The identification of these impurities was not performed.

On the other hand, the GC-MS profile of the second compound triphenyltin chloride (TPT), indicated the presence of 2 GC peaks at the retention times Rt 8.76 min

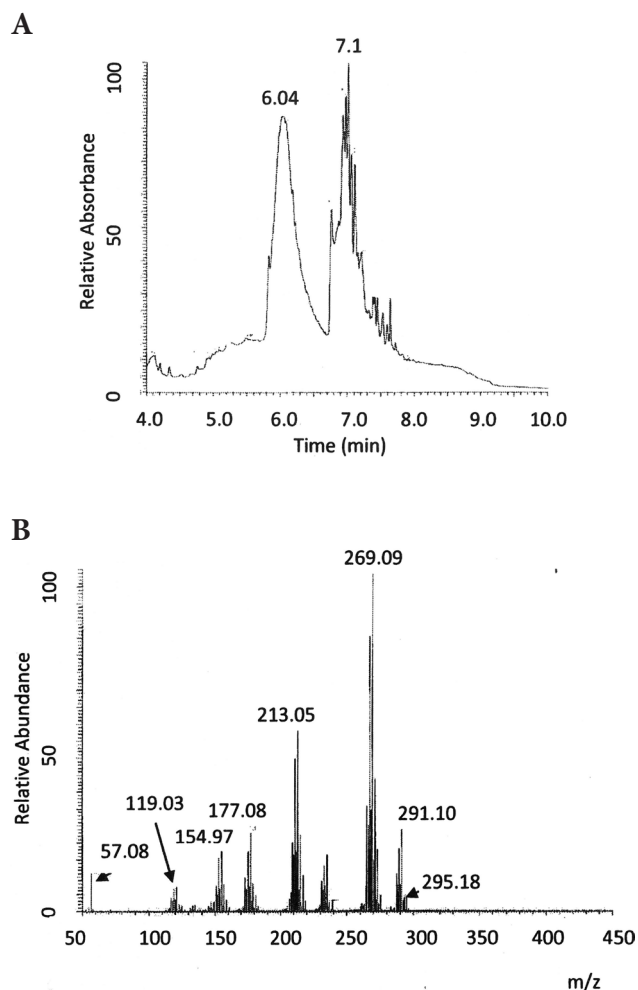


Figure 2. GC chromatogram (A) and MS spectrum (B) of commercial tributyltin chloride. A: Peak RT (retention time) 6.04 min, tributyltin chloride; RT 7.01 min, impurities.

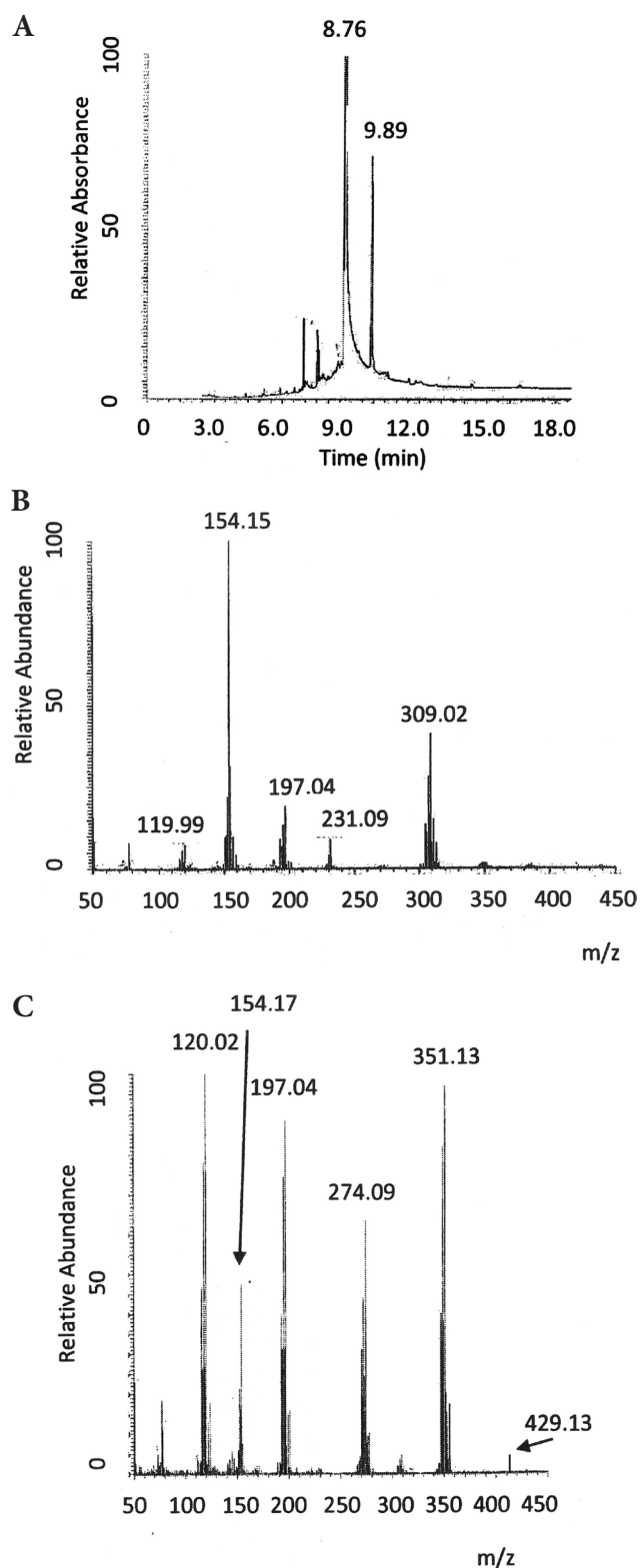


Figure 3. GC chromatogram of commercial triphenyltin chloride (A) and MS spectrum of triphenyltin chloride (B) and of impurity – tetraphenyltin (C). RT 8.76 min, triphenyltin chloride; RT 9.89 min, tetraphenyltin.

and 9.89 min (Fig. 3A) that referred to chlorotriphenyltin chloride (m/z 386, 309, 231, 197, 154, 120) (Fig. 3B) and tetraphenyltin (m/z 428, 351, 197 – this peak seems to be identical to the peak of 196 from the spectra of triphenyltin chloride) (Fig. 3C). The peak with m/z 120 – tin, also appears and is identical to the peak from the MS spectrum of triphenyltin chloride – see Fig. 3B). The presence of the impurities is also in an agreement with declared-by-the-producer purity of 96%.

The MS data served as a base for designing the fragmentation process of both investigated substances (Figs. 4A and 4B) and one impurity (tetraphenyltin) (Fig. 4C).

UPLC

By referring to the chromatograms, each compound exhibited well-resolved peaks at retention times between 0.49 min (tributyltin chloride) (Fig. 5A) and 0.56 min (triphenyltin chloride) (Fig. 6A).

Significant stability of tributyltin chloride was observed after 60 and even after 180 days at laboratory temperature. One extra peak at retention time = 0.415 min increased in size indicating the degradation of tributyltin chloride (Fig. 5B). The degradation of tributyltin chloride in a solution of artificial sea water kept at laboratory temperature for 6 months was approximately 13% of the original concentration of tributyltin chloride.

The area values of triphenyltin chloride showed excellent stability over a two and six month periods. No specific degradation peak was detected (compare Figs. 6A and 6B).

The degradation parameters (degradation constant K_{deg} and half-life time $t_{1/2}$) of both compounds were calculated using linear regression analysis. Tributyltin chloride was the less stable from both compounds with $K_{\text{deg}} = 0.00014/\text{day}$ and $t_{1/2} = 4950$ days (13.6 years). K_{deg} of the more stable triphenyltin was determined to be $K_{\text{deg}} = 0.00006/\text{day}$ with $t_{1/2} = 11550$ days (31.6 years).

Discussion

Triorganotin substances are very stable because of their chemical nature. Investigation of the rates of their degradation is very important as it may elucidate expectation on their degradation in sea water and also in sediments. Their synthesis is difficult and, consequently, their declared purity of commercial products is never close to 100%. However, this does not represent a problem as their main use is as antifouling substances for ships and ports protection. In this work, we looked into the stability of two substances – tributyltin and triphenyltin chlorides (Fig. 1) as they represent two main groups of tin products, i.e. butyl substituted and phenyl substituted. Application

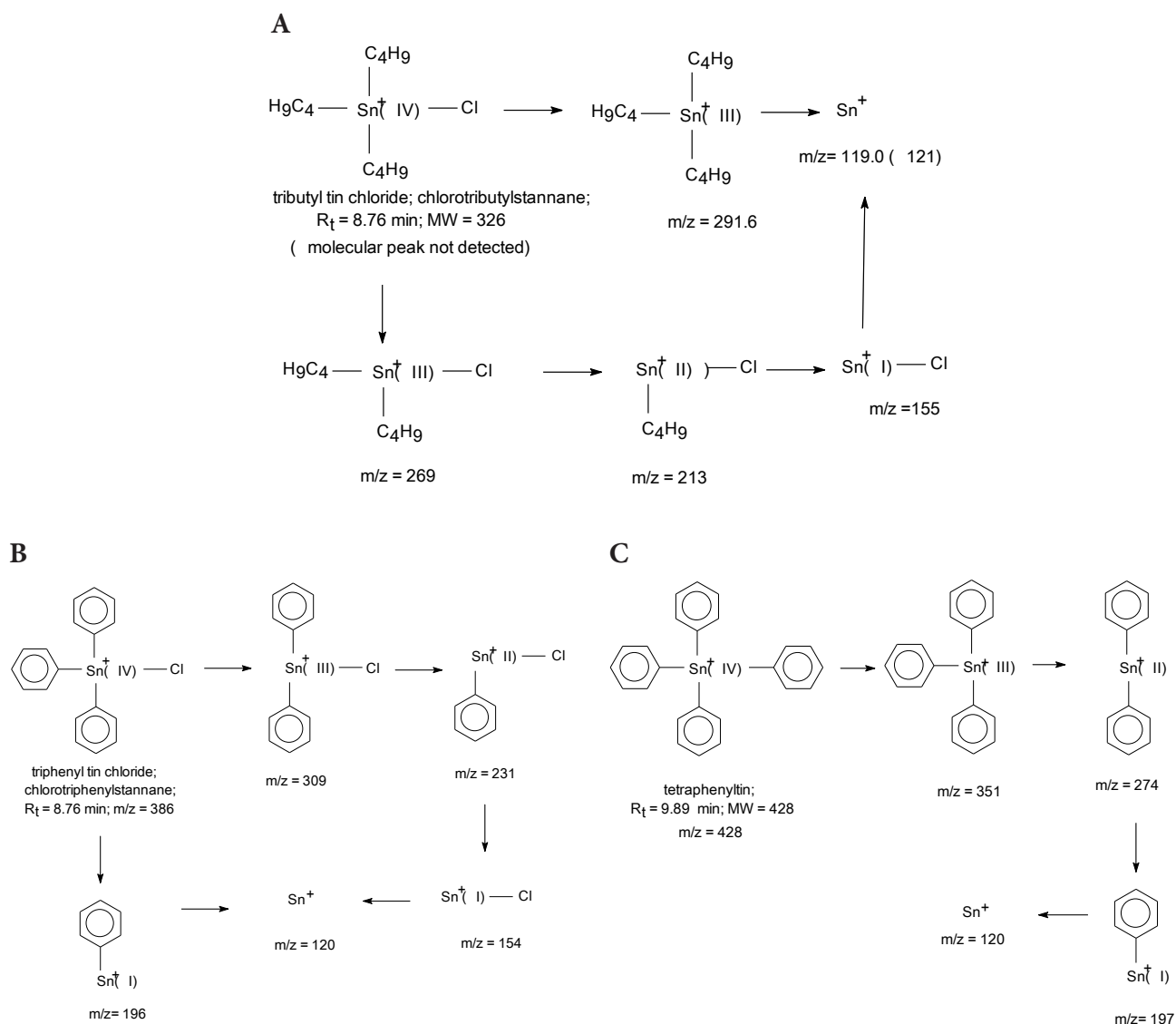


Figure 4. MS fragmentation of tributyltin chloride (A), triphenyltin chloride (B) and tetraphenyltin (C).

of modern instrumental analytical technique, UPLC, represents a novel use of this technique. This is important due to the fact that the presence of the substances investigated in the marine environment was established and consequently, their negative biological and toxic effects can take place. Additionally, the use of modern sensitive and selective instrumental technique for their study is very important as it makes monitoring of marine environment and determination more reliable, economical and suitable for routine determinations. As shown earlier, data on the availability of organotins in the environment are available (Arp et al. 2014; Rodriguez-Cea et al. 2015; Suzdalev et al. 2015). However, the importance of our study is based on non-availability of data on triorganotin degradation/stabil-

ity of these substances. For the first time, this manuscript conveys information on the stability of two organotins in an artificial sea water that mimics conditions in sea and ocean environment

Our study is complemented by GC-MS spectra that confirmed the presence of approximately 4% of impurities in tested substances. The fragmentations of the triorganotin chlorides tested and also of tetraphenyltin was designed.

The high stability of both tested compounds with half-life times for tributyltin chloride being 13.6 years and for triphenyltin chloride 31.6 years indicates a significant environmental problem when these substances enter sea water and sediments. Based on the chemical structure, it is reasonable to

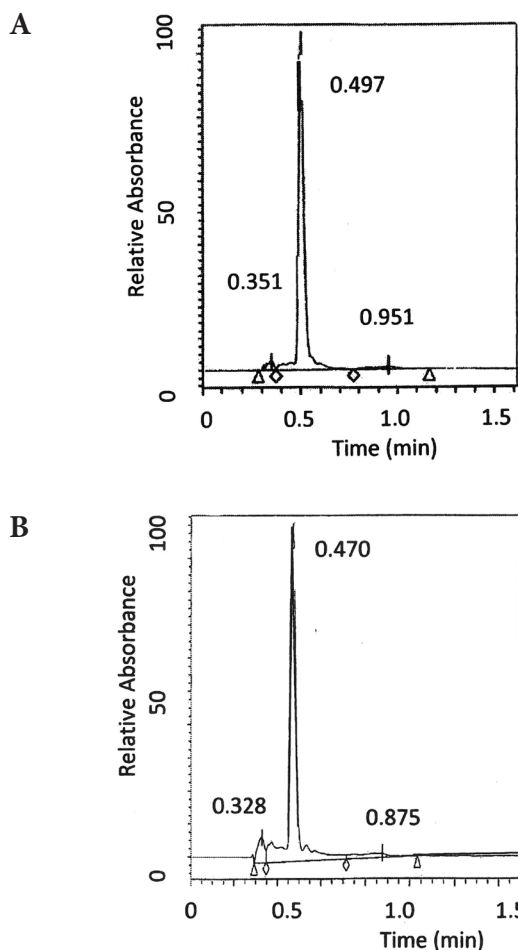


Figure 5. UPLC chromatogram of tributyltin chloride in solution at zero time (**A**) and after 180 days at room temperature (**B**). A: Peak RT (retention time) 0.351 min, area 15041 RA; RT 0.497 min, area 443735 RA; RT 0.951 min, area 16563 RA. B: Peak RT 0.328 min, area 33868 RA; RT 0.470 min, area 427382 RA; RT 0.875 min, area 30019 RA. (Area of peaks shown as “relative absorbance units”, RA).

expect that the triphenyltin chloride impurity – tetraphenyltin – is even more stable. On the other hand, the contribution of other environmental factors, such as UV radiation or presence of various microorganisms, may probably contribute to the higher rate of degradation of triorganotin. However, tin will still remain as a toxic pollutant in the environment after any of organic tin substance degradation.

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Conflict of interest. The authors declare that there is no conflict of interest.

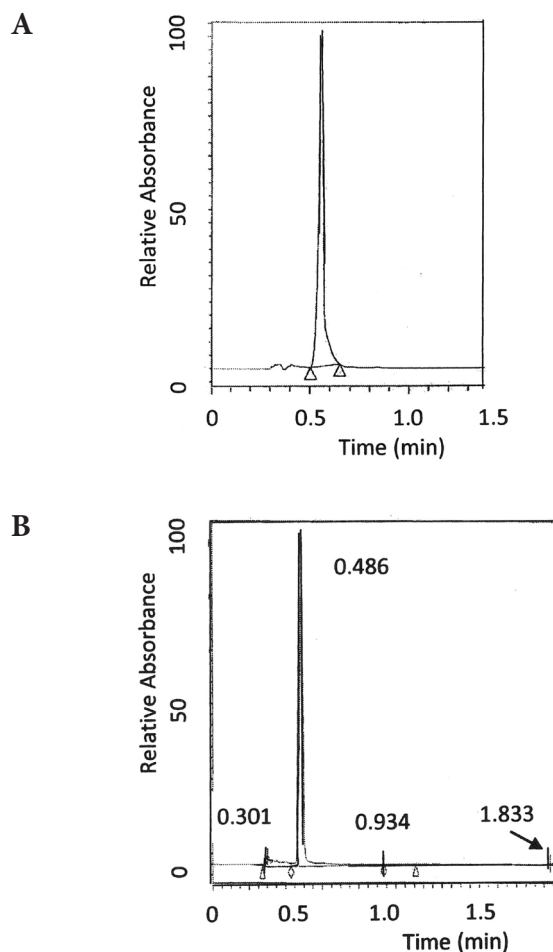


Figure 6. UPLC chromatogram of triphenyltin chloride in solution at zero time (**A**) and after 6 months at room temperature (**B**). A: Peak RT (retention time) 0.557 min, area 866513 RA. B: Peak RT 0.301 min, area 90185 RA; RT 0.486 min, area 854035 RA; RT 0.934 min, area 5247 RA; RT 1.833 min, area 13030 RA. (Area of peaks shown as “relative absorbance units”, RA).

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