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Organotins in North Sea brown shrimp (*Crangon crangon* L.) after implementation of the TBT ban

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ABSTRACT

The organotin (OT) compounds tributyltin (TBT) and triphenyltin (TPhT) are potent biocides that have been used ubiquitously in antifouling paints and pesticides since the mid-1970s. These biocides are extremely toxic to marine life, particularly marine gastropod populations. The European Union therefore took measures to reduce the use of TBT-based antifouling paints on ships and ultimately banned these paints in 2003. Despite sufficient data on OT concentrations in marine gastropods, data are scarce for other species such as the North Sea brown shrimp (*Crangon crangon*), a dominant crustacean species in North Sea inshore benthic communities. The present study provides the first spatial overview of OT concentrations in North Sea brown shrimp. We have compared these data with historical concentrations in shrimp as well as with sediment concentrations. We have also addressed the effect on the shrimp stock and any human health risks associated with the OT concentrations found. TBT and TPhT in shrimp tail muscle ranged from 4 to 124 and from 1 to 24 μg kg^{-1} DW, respectively. High levels are accumulated in estuarine areas and are clearly related with sediment concentrations (biota-sediment accumulation factor ~10). Levels have decreased approximately 10-fold since the ban took effect, coinciding with a recovery of the shrimp stock after 30 years of gradual regression. Furthermore, the OT levels found in brown shrimp no longer present a human health risk.

1. Introduction

Organotins (OTs), generally represented by the formula R_XSnL_{4-X}, contain a tetravalent tin atom (Sn) covalently bound to 1–4 organic substituents (R; e.g., $-CH_3$, $-C_4H_9$, $-C_5H_5$ and $-C_8H_{17}$) and 1–3 halogen atoms or oxygen- or sulfur-based organic moieties (L; e.g., –Cl, –F, –SR′ and –OR′). Owing to their strong biocidal activity, tributyltin (TBT, $(C_4H_9)_3SnL$) and triphenyltin (TPhT, $(C_5H_5)_3SnL$) have been widely used in pest control. TPhT ("fentin") has been a popular fungicide in potato, sugar beet and hop culture (Hoch, 2001). In 2003, the EU banned the use of fentin because of serious concerns about operator safety and undesired effects on non-target organisms.

Starting in the mid-1970s, TBT was the compound of choice (and to a lesser extent TPhT) in antifouling agents to reduce drag or damage on ship hulls, buoys, fish nets and cages due to the attachment of fouling organisms (e.g., barnacles, algae). At the end of the 1970s, an undesired impact of TBT on the calcification of oyster shells was observed (Alzieu et al., 1982). Soon thereafter, the relationship between TBT and imposex in marine snails was discovered (Smith, 1981). France was the first country to respond by issuing a ban on the application of OT-based antifouling paints on hulls of ships smaller than 25 m in 1982. Similar bans throughout the North Sea countries followed between 1987 and 1991. In 1989, the EU imposed these measures on all Member States through Directive 89/677/EEC. In 2001, the International Maritime Organization (IMO) adopted the 'International Convention on the Control of Harmful Antifouling Systems' (AFS Convention), which prohibited the application of OTs as antifouling agents. The AFS Convention entered into force on 17 September 2007 and globally banned OTs on marine vessels starting on 17 September 2008. The EU transposed the AFS Convention into Regulation (EC) 782/2003, which banned the application of organotin on EU-flagged vessels starting on 1 January 2003. That regulation further obliged all ships visiting EU ports from 1 January 2008 onto be free of OTs or to at least bear a barrier coating. These measures have led to a slow recovery of TBT-sensitive gastropod populations and their TBT-related imposex

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prevalence is decreasing (Morton, 2009; Oliveira et al., 2009; Rodríguez et al., 2009; Sousa et al., 2009).

Brown shrimp (*Crangon crangon* L.) has a wide geographical distribution, ranging from the Icelandic coast and the White Sea (North-West Russia) to the Moroccan coast and the Black Sea. It dominates the shallow sandy coastal areas of the southern North Sea and the associated estuaries, from northern France to Denmark, where brown shrimp can comprise up to 80% of the total benthic biomass (Bamber and Henderson, 1994; Cattrijsse et al., 1997). In the associated benthic ecosystem, *C. crangon* has a pivotal function; it is both a prominent predator of juvenile fish and smaller invertebrate species (Pihl and Rosenberg, 1984; Wennhage and Gibson, 1998; Hiddink et al., 2002; Wennhage, 2002) and is also crucial prey for economically important fish species such as cod and whiting. *C. crangon* also has direct economic importance, with an annual catch of almost 40 000 tonnes and a third-place ranking in landings value of the North Sea Fisheries (ICES, 2010). The present study contains data on TBT and TPhT concentrations in *C. crangon* caught between 1 September and 10 November 2009 at multiple sampling stations from De Panne (Belgium) to Esbjerg (Denmark). These stations represent the major commercial fishing grounds. We have focused on the Westerschelde, a heavily polluted European estuary associated with one of the most densely populated areas (Flanders) and largest ports (Antwerp) in the world. The Westerschelde has a large *C. crangon* population and hosts an important nursery ground, the estuarine tidal marsh "Verdronken Land Van Saeftinghe" (Cattrijsse et al., 1997). Associated biota-sediment accumulation factors (BSAFs) were derived. Finally, we have studied the effects of the 2008 global TBT ban on the OT content and status of the Southern Bight shrimp stock and associated human health risks.

2. Experimental

2.1. Sample collection

Samples were collected during 1 September–10 November, 2009 during the Dutch, German and Belgian Demersal (Young) Fish Surveys (DFS in the Netherlands, DYFS in Germany and Belgium) performed by the Institute for Marine Resources and Ecosystem Studies (IMARES, Netherlands), Johann Heinrich von Thünen-Institute, Institute of Sea Fisheries (vTI-SF, Germany) and the Institute for Agricultural and Fisheries Research (ILVO, Belgium) (Supplementary Material, Table 1). Samples were immediately frozen aboard and stored at −20 °C at the related institutes prior to transportation to ILVO (Ostend, Belgium) in polystyrene foam insulated containers. Further sample preparation, extraction, clean-up, gas chromatographic (GC) analyses and quality control were done in accordance with the guidance for monitoring of organotin levels in marine biota (Monteyne et al., 2010) and were performed at the marine chemistry lab (MARCHEM) of MUMM in Ostend, which is accredited in accordance with the recognized International Standard ISO/IEC 17025:2005.

2.2. Sample preparation

Shrimp were allowed to thaw overnight, then were peeled and briefly rinsed with ultrapure water to obtain approximately 100 g of tail muscle for each sampling station. Samples were mixed in borosilicate petri dishes using a rotor/stator type homogenizer (Ultraturrax T25 basic, IKA-Labortechnik GmbH, Staufen, Germany), freeze-dried with a Christ LMC-2 (Martin *Christ* Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) lyophilizer and pulverized manually using a porcelain mortar and pestle. The powder was weighed for calculation of dry weight (DW) – wet weight (WW) ratio, and was stored in a desiccator cabinet until analysis.

2.3. Sample extraction and clean-up

The procedure for OT extraction was based on the use of acid reagents in methanol and stirring in hexane. About 1 g of shrimp powder was transferred into an amber 40 mL screw cap vial, and then l5 mL methanol and 7 mL hexane were added. Samples were buffered to pH 5 by adding 3 mL of 4 M sodium acetate. An aliquot of 25 μL of tripropyltin solution (10 μg Sn/g in methanol) was added as recovery standard prior to derivatization for QA/QC purposes to check the ethylation efficacy of TBT. Ethylation was combined with a continuous desorption process by drop-by-drop addition of 4 mL of sodium tetraethyl borate (Sigma–Aldrich, Steinheim, Germany) prepared with deionized water (5%, *v/v*) to the samples while vigorously stirring. For the degradation of boroxin formed due to the intensive derivation (Smedes et al., 2000), an aliquot of 5 mL of 10 M sodium hydroxide was added to the samples. Finally, the internal standards tetrapropyltin (used for quantification) and pentyltriphenyltin (additional standard for QA/QC purpose) of a concentration of 4 μg Sn/g in hexane were added to the samples, and the phases were separated by centrifugation. The purity of all solvents was appropriate for organic residue analysis. Chlorinated and ethylated OTs were obtained from QUASIMEME (Wageningen, the Netherlands). Internal standard and recovery standard tetrapropyltin and tripropyltin chloride were purchased from Schmidt (Amsterdam, the Netherlands). Glassware was washed with 10% hydrochloric acid and rinsed six times with ultrapure water. Custom-made chromatography columns (200 mm × 9 mm ID) were filled with 2 g of florisil (Merck, Darmstadt, Germany) and 25 mL of hexane was used as the clean-up elution. The extracts were stored at 4 °C until GC analysis.

2.4. Gas chromatographic analysis

A large-volume injection (LVI) technique was developed (Monteyne et al., unpublished protocol). Fifty microliters of sample was injected by an autosampler (Combipal, CTC Analytics, Italy) at a rate of 1.7 μL s^{-1} through a Programmed Temperature Vaporizing (PTV) injector (Thermo Electron Corporation, Austin, TX, USA), using a glass sintered liner. The analytic system consisted of a Trace GC (ThermoQuest, Milan, Italy), a 20 m Rtx®-5 SILMS analytical column (0.25 mm ID) with a 5% phenyl polysilphenylene-siloxane stationary phase (0.25 μm film thickness; Restek, Bellefonte, PA, USA). The oven was preheated to 35 °C and maintained that temperature for 4 min, after which the temperature was increased at the rate of 20 °C min^{-1} to 120 °C (ramp 1), at a rate of 7 °C min^{-1} to 150 °C (ramp 2) and finally at a rate of 20 °C min^{-1} to 300 °C (ramp 3) (5 min hold). A carrier flow of helium of 1.5 mL min^{-1} was used. The compounds were detected by a Finnigan Trace MS in electron-impact ionization (EI) mode operating in selected ion monitoring (SIM).

2.5. Quality control

Multi-level calibration curves ($r^2 > 0.995$) in the linear response interval of the detector were created for quantification. The identification was based on retention times and intensity ratios of 3 monitored ions for quantification. The quality control was performed by regular analysis of procedural blanks, a procedural spike of 100 ng Sn/g, duplo measurements, internal reference material (mussel tissue) and certified reference material (mussel tissue ERM®-CE477). Recovery of MBT, DBT and TBT in ERM®-CE477 was 117 ± 14%, 97 ± 15% and 99 ± 11% (n = 12, 4-year period), respectively. Recovery of TPhT in the procedural spike is 90 ± 15%. Semi-annual international proficiency test (QUASIMEME) results were also consistently successful. Limits of quantification (LoQ) for TBT and TPhT were 1 μg kg^{-1} DW. LoQs of monobutyltin (MBT), dibutyltin