Marine Environmental Research 76 (2012) 56–62

Contents lists available at SciVerse ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Specific accumulation of organotin compounds in tissues of the rock shell, *Thais clavigera*

Toshihiro Horiguchi[a,*], Jeong-Hoon Lee[a], Jeong-Chae Park[a,b], Hyeon-Seo Cho[c], Hiroaki Shiraishi[a], Masatoshi Morita[d]

[a] *Center for Environmental Risk Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan*
[b] *The United Graduate School of Veterinary Science, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8511, Japan*
[c] *Faculty of Marine Technology, College of Ocean Science and Technology, Chonnam National University, San 96-1, Dundeok-dong, Yeosu 550-749, Republic of Korea*
[d] *3-5-7 Tarumi, Faculty of Agriculture, Ehime University, Matsuyama, Ehime 790-8566, Japan*

ARTICLE INFO

Article history:
Received 30 May 2011
Received in revised form
14 August 2011
Accepted 8 September 2011

Keywords:
Imposex
Rock shell (*Thais clavigera*)
Tributyltin (TBT)
Triphenyltin (TPT)
Accumulation
Tissue distribution
Retinoid X receptor (RXR)

ABSTRACT

Concentrations of organotin compounds (butyltins and phenyltins) were determined in gonad, accessory sex organs, penis, digestive gland, kidney, radula with sac, oesophagus with crop, stomach, hypobranchial gland, rectum, mantle, osphradium, ctenidium, heart, salivary gland, head ganglia and muscle of imposex-exhibiting female and male rock shells (*Thais clavigera*), by gas chromatography with flame photometric detection (GC-FPD). Different tissue distributions were observed between butyltin and phenyltin compounds. More than 1000 ng TBT/g wet wt. were observed in ovary, digestive gland, kidney, heart, ctenidium, osphradium, stomach, head ganglia and penis, of both imposex-exhibiting females and males. More than 1000 ng TPT/g wet wt. were found in almost all tissues of both sexes. Approximately one-third or more of total TBT was accumulated in the digestive glands of both females and males, respectively. Meanwhile, approximately 40–50% and one-half of total TPT accumulated in the digestive glands of females and males, respectively.

1. Introduction

Organotin-based antifouling paints are very effective in preventing the settling of barnacles, oysters, and other sessile organisms on the hulls of vessels and the surfaces of fishing nets. This effectiveness, however, means that organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), are strongly toxic to aquatic organisms. There are numerous papers and several reviews on the toxicities of TBT and TPT to aquatic invertebrates and vertebrates (e.g., reviews by Alzieu, 1996; Hall and Bushong, 1996). Lethal, developmental, behavioral, and reproductive toxicities as well as other various kinds of physiological toxic effects have been reported in aquatic invertebrates and vertebrates.

One of the typical adverse effects caused by organotin compounds, such as TBT and TPT, in aquatic organisms is imposex, represented by masculinization of females, in prosobranch gastropod mollusks (e.g., Gibbs and Bryan, 1986, 1996; Horiguchi, 2009a, b). The term imposex was coined by Smith (1971) to describe the syndrome of superimposition of male-type genital organs, such as the penis and vas deferens, on female gastropods. Imposex is thought to be irreversible (Bryan et al. (1986)). Reproductive failure may occur in females with severe imposex, resulting in population decline or even mass extinction (Gibbs and Bryan, 1986, 1996). In some species, imposex is typically induced by TBT and TPT from antifouling paints used on ship hulls and fishing nets (Bryan et al., 1987, 1988; Gibbs et al., 1987; Horiguchi et al., 1995, 1997). As of 2005, approximately 200 species of mesogastropods and neogastropods had been reported to be affected by imposex worldwide (Bech, 2002a,b; Fioroni et al., 1991; Horiguchi et al., 1997b; Marshall and Rajkumar, 2003; Shi et al., 2005; Sole et al., 1998; ten Hallers-Tjabbes et al., 2003; Terlizzi et al., 2004); many of these gastropod species belong to the families Muricidae (e.g., *Nucella lapillus*, *Ocenebra erinacea*, *Thais clavigera,* and *Urosalpinx cinerea*), Buccinidae (e.g., *Babylonia japonica*, *Buccinum undatum*, and *Neptunea arthritica arthritica*), Conidae (e.g., *Conus marmoreus bandanus* and *Virroconus ebraeus*), and Nassariidae (e.g., *Ilyanassa obsoleta* and *Nassarius reticulatus*) of the Neogastropoda (Fioroni et al., 1991; Horiguchi et al., 1997b).

Abbreviations: DBT, dibutyltin; GC-FPD, gas chromatography with flame photometric detection; MBT, monobutyltin; RXR, retinoid X receptor; TBT, tributyltin; TPT, triphenyltin.

* Corresponding author. Tel.: +81 29 850 2522; fax: +81 29 850 2870.
E-mail address: thorigu@nies.go.jp (T. Horiguchi).

0141-1136/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2011.09.002

Regarding the mechanism by which organotins, such as TBT and TPT, induce the development of imposex in gastropods, six hypotheses have been proposed: (1) an increase in androgen (e.g., testosterone) levels as a result of TBT-mediated inhibition of aromatase (Bettin et al., 1996); (2) an increase in testosterone levels owing to the inhibition of acyl CoA-steroid acyltransferase (Gooding et al., 2003; Sternberg and LeBlanc, 2006); (3) TBT-mediated inhibition of the excretion of androgen sulfate conjugates, with a consequent increase in androgen levels (Ronis and Mason, 1996); (4) TBT interference with the release of penis morphogenetic/retrogressive factor from the pedal/cerebropleural ganglia (Féral and Le Gall, 1983); (5) an increase in the level of an alanine-proline-glycine-tryptophan amide neuropeptide in response to TBT (Oberdörster and McClellan-Green, 2000); and (6) activation of the retinoid X receptor (RXR) (Nishikawa et al., 2004). Although scientific debate is still continuing, there are several papers in which a hypothesis of activation of RXR is supported (Castro et al., 2007; Horiguchi et al., 2007, 2008, 2010a, b; Sternberg et al., 2008; Urushitani et al., 2011).

Information about detailed tissue distribution of organotin compounds, such as TBT and TPT, is useful for elucidation of the mechanism of imposex induced by TBT and/or TPT in gastropods. Nevertheless, less is known about detailed tissue distribution of organotin compounds, such as TBT and TPT, in gastropods. Here, we determined concentrations of organotins, such as TBT, TPT and their metabolites, in various tissues of the rock shell, *T. clavigera*, by gas chromatography with flame photometric detection (GC-FPD) to examine distributions of butyltins and phenyltins in tissues of *T. clavigera* and to discuss the mode of action of TBT and/or TPT in the development of imposex in gastropods.

2. Materials and methods

2.1. Collection, sex determination, imposex identification and tissue separation of T. clavigera specimens

Specimens of *T. clavigera* were collected at Jogashima (35°08′ 02.63″ N, 139°37′ 37.21″ E: Fig. 1), Kanagawa Prefecture, one of the most contaminated sites by organotin compounds in Japan, on June 18, 1996. After the collection, they were kept in freezer of − 20 °C until anatomical observation for imposex and chemical analysis for determination of organotins in tissue. The frozen specimens ($n = 40$) were defrosted and then sex determination was perfomed according to existence of female accessory sex organs, such as albumen, sperm-ingesting and capsule glands (Horiguchi et al., 1994). Sex ratio of females and males used in this study was 22:18, and size of the specimens is shown in Table 1.

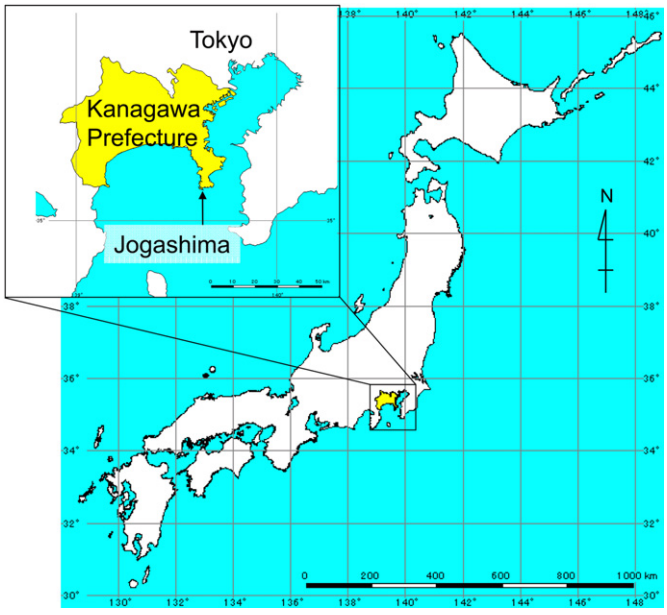

Fig. 1. Sampling sites of the rock shell (*Thais clavigera*) specimens.

Imposex was also evaluated in females, namely, percentage occurence of imposex, penis length, development of vas deferens and oviduct blockage (i.e., sterile individual) (Horiguchi et al., 1994). Male penis length was also measured.

Gonad (ovary or testis), accessory sex organs (albumen, sperm-ingesting and capsule glands or prostate gland), penis, digestive gland, kidney, radula with sac, oesophagus with crop, stomach, hypobranchial gland, rectum, mantle, osphradium, ctenidium, heart, salivary gland, head ganglia and muscle [foot, siphon, head with tentacle and others] of imposex-exhibiting female and male *T. clavigera* were separated/removed.

2.2. Chemical analysis of organotin compounds in tissues of T. clavigera specimens

Chemical analyses of butyltin and phenyltin compounds in tissues (gonad (ovary or testis), accessory sex organs (albumen, sperm-ingesting and capsule glands or prostate gland), penis, digestive gland, kidney, radula with sac, oesophagus with crop, stomach, hypobranchial gland, rectum, mantle, osphradium, ctenidium, heart, salivary gland, head ganglia and muscle [foot, siphon, head with tentacle and others]) of *T. clavigera* specimens were conducted with composite samples using the method described in Horiguchi et al. (1994). Briefly, tissues were extracted with 0.1% tropolone/benzene and 1 N hydrobromic acid/ethanol by ultrasonication, derivatized with propylmagnesium bromide, cleaned by silica gel column chromatography, and quantified by gas chromatography with flame photometric detection (GC-FPD). The detection limit of the instrument was 50 pg, and certified reference material from Japanese sea bass (*Lateolabrax japonicus*) for TBT and TPT analysis (prepared by the National Institute for Environmental Studies; NIES CRM no. 11) was used for quality assurance and quality control. The analytical conditions are described in more detail in Horiguchi et al. (1994).

3. Results

3.1. Imposex parameters

Percentage occurence of imposex was 100% in female specimens. Female penis length was rather long and almost equal to male's one (Table 2). Vas deferens was also well developed in females (i.e., imposex-exhibiting individuals), and 91.0% of females (i.e., imposex-exhibiting females) had oviducts blocked involved by vas deferens formation, which were recognized as sterile individuals (Table 2).

Table 1
Body sizes of *T. clavigera* specimens used in this study.

	Females[a]	Males[a]
Shell height (mm)	31.9 ± 3.2	31.3 ± 3.8
Shell width (mm)	20.8 ± 2.0	20.7 ± 2.2
Shell weight (g)	5.57 ± 1.66	5.56 ± 1.76
Soft tissue weight (g)	2.02 ± 0.50	1.97 ± 0.68

$N = 40$; female: male = 22:18.
[a] Shown as mean ± standard deviation.