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Tributyltin: Advancing the Science on Assessing Endocrine Disruption with an Unconventional Endocrine-Disrupting Compound

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1 Introduction

Tributyltin (TBT) was introduced as a biocide in the 1960s and today its use is widely restricted by a variety of statutes. Although some countries (and local governments within those countries) restricted the use of TBT in antifouling paints as early as 1982 (Alzieu 2000; Champ 2000), it was not until 2008 that a more global ban was enacted. In that year, the International Convention on the Control of Harmful Antifouling Systems for Ships required its signatories to ensure that vessels would no longer use hull paint containing TBT or other organotin chemicals. This agreement was signed by 74 countries as of the end of October 2016. Unfortunately, a recent study has confirmed that recreational vessels sampled from countries around the Baltic Sea still contain high concentrations of TBT and triphenyltin (TPT) and may be a source to the environment (Lagerström et al. 2016).

The main inputs of tributyltin into the environment are contaminated water and sediment originating from ports, harbors, marinas, and boat yards due to leaching from boat paint and improper disposal. Contaminated sediment can be mobilized by dredging, bioturbation, ship scour, or weather events and TBT-contaminated water can be carried by currents to previously unimpacted locations. Tributyltin may also be introduced into the environment by ongoing release from previously treated

structures (continuous release and during cleaning/renovation) and small-scale (mis-)use or disposal of prohibited antifouling products. Diffuse exposure may also arise from the use or disposal of previously TBT-treated wooden articles and other applications including its use as a stabilizer for PVC products, antifungal treatment, and as a preservative for wood, paper, and textiles (US EPA 2003).

Tributyltin may be one of the most toxic man-made chemicals ever intentionally released into the environment, eliciting endocrine-type responses at concentrations in the range of 1 ng/L for water or 10 ng/g for whole body. Even concentrations in the range of low μg/L aqueous or low μg/g tissue cause high rates of mortality in many species. However, outright mortality events due to TBT exposure are relatively rare. Therefore, the most important environmental consequences result from sublethal responses. Among them, the development of male sexual characteristics in female marine gastropods exposed to TBT, a phenomenon known as imposex, has been abundantly documented (e.g., Gibbs and Bryan 1996a). This abnormality has resulted in reproductive failure of populations of Caenogastropods globally, leading to mass extinction and subsequent alterations in community structure and functioning of coastal ecosystems (Gibbs and Bryan 1986, 1996b; Hawkins et al. 1994).

The main goal for this review was to examine the available literature on TBT as an EDC and provide a synopsis on population-relevant responses across major taxa. Additionally, we highlight the case of TBT as an unusual endocrine disruptor and discuss some of the reasons why its toxic potential and MeOA went unrealized for many years. Finally, we use the Organization for Economic Co-operation and Development (OECD) Conceptual Framework for Endocrine Disruptor Testing and Assessment to organize the available information on effect assessment and environmental exposure levels to conduct a tentative retrospective environmental risk assessment of TBT.

2 Methods

2.1 Literature Search and Selection of Data

To conduct this review, 160 references were selected from an initial list of approximately 965 regulatory reports and open and grey literature, in an attempt to capture relevant data from original studies on fish (45 references), molluscs (55 references), and other taxonomic groups including mammals (60 references); these were sorted according to the type of effect. This was not intended to be an exhaustive review of the extensive TBT literature, so it is possible that some relevant studies were inadvertently omitted.

2.2 Quality Evaluation of Relevant Data

TBT is a data-rich compound with numerous tests at various levels of biological organization. However, the vast majority of these studies did not follow

international standardized test guidelines. Nevertheless, several full or partial life-cycle tests are available for mammals, fish, and several invertebrate taxa. Data previously validated for regulatory reviews (e.g., EU 2005; US EPA 2003, 2008) were assumed reliable; however, as far as possible, original studies were used as sources of data. Other ecotoxicity studies were quality checked and scored using Klimisch scores (e.g., using the ToxRTool: https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/toxrtool). Only studies ranked as Klimisch 1 and 2 were used for the subsequent analysis (although a small number of papers ranked as Klimisch 3 or 4 were used as supporting information if their findings were verified by other studies). However, Klimisch scores do not apply to field studies, which were a major source of data. Similarly, histopathological investigations are difficult to evaluate using these criteria. Therefore, expert judgment was used to evaluate the validity/credibility of these studies.

Many laboratory studies were performed using static or semi-static exposure regimes, and the reported effect concentrations were frequently based on nominal concentrations, due to a lack of analytical verification of the test concentrations over the study duration. TBT is adsorptive and test concentrations in non-flow-through studies are likely to be highly variable. This also means that equipment can easily be contaminated, thus affecting the actual exposure concentrations. In addition, a variety of units have been used to express the TBT concentration in the literature. To aid comparisons between studies, concentrations are expressed hereafter in a common unit (TBT ion). However, for simplicity and since the difference in molecular weight is small, any concentrations reported as TBTO (bis(tributyltin) oxide) or TBTCl (tributyltin chloride) were assumed to be effectively the same as TBT. Conversion to TBT was therefore made when the unit was originally expressed in terms of tin (Sn) and was accomplished by applying a factor of 2.44, which is the difference in molecular weight.

Other important limitations of the data included wide spacing between test concentrations and consequently between the LOEC and NOEC in some laboratory studies (very few report data in terms of EC_x values), poor methodological descriptions/statistical analyses, and uncertainties in the association between reported dissolved concentrations and observed effects in field studies (since the concentration at the time of adverse event initiation may be different, and organisms may accumulate TBT over a long period).

3 Environmental Fate and Occurrence

3.1 Physical and Chemical Properties

Several tributyltin compounds exist, but in general they all rapidly dissociate in water to form the tributyltin (TBT) cation, which is the toxic moiety. An example is bis(tributyltin) oxide ("tributyltin oxide" or TBTO), which has a vapor pressure of less than 0.016 Pa at 20 °C, a water solubility in the range 0.7–71 mg/L at 20 °C

(depending on pH), and an octanol-water partition coefficient (K_{ow}) around 3.5 (as a log value) (e.g., ECHA 2008; EU 2005; US EPA 2003). The pH-specific K_{ow} (D_{ow}) for TBT is constant above pH 6 (Arnold et al. 1997). An organic carbon-water partition coefficient of 32,000 ($\log_{10} K_{oc} = 4.5$) was proposed by Meador (2000).

3.2 Uses

Historically, TBT compounds were widely used as biocides in antifouling products, parasite control products, and wood preservatives. Regulatory controls have been implemented in many jurisdictions (e.g., under the International Maritime Organization's Anti-Fouling Systems Convention), but some residual biocidal uses in these and other types of industrial and consumer products (including sports clothing) may still occur in some parts of the world (Antizar-Ladislao 2008; Choudhury 2014). They are also used as chemical intermediates for the production of other organotins (e.g., dibutyltin stabilizers for PVC), and may therefore occur as unintentional impurities (e.g., OECD 2007). However, these uses are declining.

3.3 Metabolism

TBT can be metabolized sequentially by cytochrome P450 (CYP450) enzymes to dibutyl- and monobutyltin (e.g., Cooke et al. 2008; Strand et al. 2009). Metabolic capabilities vary widely among taxa (e.g., Ohhira et al. 2003, 2006a, b; Bartlett et al. 2007; Oehlmann et al. 2007; Yang et al. 2009) and even between sexes in mammals (Ohhira et al. 2006b). High bioaccumulation levels in invertebrates and fish are believed to be due to a low capacity for metabolism/excretion and high rates of uptake (Meador 1997).

3.4 Potential Exposure Routes

TBT has a degradation half-life of days to months in water and up to several years in sediment (e.g., ECHA 2008). It is very bioaccumulative, with whole fish bioconcentration factors in the range 2000–50,000 L/kg. The bioaccumulation potential in molluscs can be higher, but is generally similar to that for fish (e.g., Meador 2006; ECHA 2008). TBT bioaccumulation does not follow equilibrium partitioning (Meador 2000). Consequently, some fish exhibit high bioaccumulation factors, which may result from high rates of ventilation. Aquatic organisms can be exposed via both the water column and ingestion of contaminated food (including sediment), although there are no data to suggest that biomagnification occurs in

food webs. Terrestrial organisms may also be exposed via TBT-contaminated sediments (e.g., during flood events or disposal of sediment dredging), application of biocidal products and/or contaminated sewage sludge to soil, atmospheric deposition, and by ingestion of contaminated food or water (Antizar-Ladislao 2008; Silva et al. 2014).

3.5 *Environmental Concentrations*

For this review we did not conduct an exhaustive search for environmental concentrations, but examined past compilations. Several review articles describe the occurrence of butyltins in water from locations around the world over the past several decades (Fent 1996; Antizar-Ladislao 2008). More recent monitoring performed in English and Welsh estuarine surface waters during 2012–2014 was selected as representative of current aquatic levels in a region known to be previously contaminated (UK Environment Agency, pers. com. 2016). The dataset has a number of non-detects; however, the following information is considered to be reasonably conservative for aqueous concentrations: 90th percentile: 0.5 ng/L; arithmetic mean: 0.3 ng/L; median: 0.2 ng/L; range: 0.1–8 ng/L ($n = 269$; two outlier values, 44 and 1368 μg/L, were removed prior to the calculation).

A recent review of tissue concentrations for biota from a variety of countries indicated relatively high TBT concentrations in molluscs, fish, aquatic birds, and marine mammals ranging from low ng/g to low μg/g concentrations, although there were indications of significant decreases with time in some species (Elliott et al. 2007; Mizukawa et al. 2009; Meador 2011).

4 Primary Molecular Initiating Event (MIE)

4.1 *In Vitro and In Silico Analyses*

Binding to a member of the nuclear receptor superfamily is a common MIE associated with endocrine and metabolic pathways. All relevant in vitro assays currently utilize mammalian receptors, assuming interspecies conservation of the structural and functional aspects of each receptor. Among those methods, ToxCast™ uses high-throughput screening methods and computational toxicology approaches to rank and prioritize chemicals. For the US EPA's Endocrine Disruption Screening Program (EDSP), the initial focus of these screening methods has been on estrogen, androgen, and thyroid (EAT) hormone interactions. Analysis of TBT using ToxCast™ identified activity in ER and AR assays at levels generally in excess of the cytotoxicity limit (Fig. 1). In addition, the EDSP21 Dashboard identified TBT as inactive for EAT screens (http://actor.epa.gov/edsp21/).

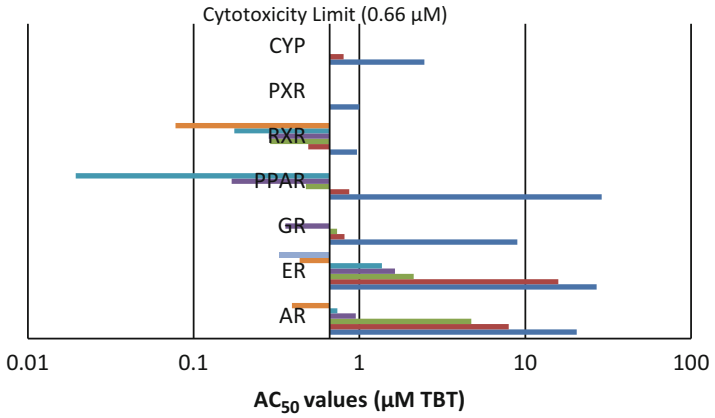


Fig. 1 Selected ToxCast™ nuclear receptor family AC_{50} data for TBT (downloaded March 2016). *CYP* Cytochrome P450, *PXR* pregnane X receptor, *RXR* retinoic X receptor, *PPAR* peroxisome proliferator-activation receptor, *GR* glucocorticoid receptor, *ER* estrogen receptor, *AR* androgen receptor

However, TBT activity in RXR and PPAR assays typically occurred at levels less than the cytotoxicity limit and at lower levels than most ER or AR assays. Thus, ToxCast™ identified the potential of TBT to act through activation of RXR and/or PPAR pathways, although TBT may have been missed as a potential EDC if screening was limited to EAT-related assays alone.

Using an RXR-permissive PPARγ reporter cell line, Grimaldi et al. (2015) demonstrated that, among other butyltins, TBT is able to activate RXR at nanomolar concentrations. Structural profiling using in silico 3D-modelling of the ligand-binding pocket (LBP) of the mollusc *Lymnaea stagnalis* RXR showed that amino-acid residues involved in the binding of RXR ligands (organotins, including TBT, and cis-9-retinoic acid) are identical between *Lymnaea* and humans (Boulahtouf et al. 2015). In addition, the RXR receptor from the freshwater mollusc *Biomphalaria glabrata* does not only bind retinoic acid, but also activates transcription (Bouton et al. 2005). Other studies on invertebrate RXRs have identified significant differences in the LBP as well as a number of mutations that result in low or no affinity of RXR for their vertebrate ligands (all retinoic acids). These include the retinoic acid receptor (RAR) from the marine snail *Nucella lapillus* (Gutierrez-Mazariegos et al. 2014) and the RXR from *Daphnia magna* (Wang et al. 2007), highlighting a knowledge gap regarding the natural ligands relevant to RXR signaling pathways in invertebrate species. We anticipate that with the increasing genomic resources for these important phyla, both the structure and the function of nuclear receptors such as RXR can be further elucidated. This will aid not only our understanding of evolutionally processes but also the risk posed by chemicals such as TBT, which can activate these important transcription factors.

4.2 Weight-of-Evidence for RXR and PPAR$_\gamma$ Pathways

4.2.1 Molluscs

From both in vivo (including injection experiments) and in vitro (transactivation assays) studies, there is strong evidence that TBT interacts with RXR in both marine (e.g., Castro et al. 2007) and freshwater (Boulahtouf et al. 2015) gastropod species. Such interactions were observed at TBT concentrations of 1 nM TBT (equiv. 290 ng TBT/L) in transactivation assays and 1000 ng TBT/g body wet weight through injection. It has also been shown that RXR gene transcription is modulated by TBT, with an increase in the penis-forming area of imposexed females (Lima et al. 2011). Hence, interaction of TBT with RXR seems to be the main molecular event initiating changes in the development of sexual organs in female snails, ultimately resulting in imposex. Importantly, imposex can be induced by cis-9-RA, the natural ligand of RXR (Nishikawa et al. 2004; Castro et al. 2007). More recently, the use of an open transcriptomic approach supported the involvement of steroid, neuroendocrine peptide hormone dysfunction and retinoid mechanisms in imposex induction by chronic exposure to TBT in *Nucella lapillus* (Pascoal et al. 2013). This study also suggested the involvement of PPAR pathways and showed that rosiglitazone, a well-known vertebrate PPAR$_\gamma$ ligand, induced imposex. Nevertheless, although it is certain that activation of RXR and/or RXR/PPAR is the MIE, the mechanistic links with subsequent pathways remain largely unexplained.

However, other primary molecular mechanisms for TBT effects have also been proposed. These include the activation of gonadotropin releasing hormone receptor (GnRHR) along with the gonadotropin releasing hormone (GnRH) (Castro et al. 2007). In *Octopus vulgaris*, it has been shown that a GnRH-like peptide contributed in vitro to an elevation of basal steroidogenesis of testosterone, progesterone, and 17β-oestradiol, in a concentration-dependent manner in both follicle and spermatozoa (Kanda et al. 2006). Another hypothesis formed around the imposex phenomenon suggested that TBT is a neurotoxicant, causing the aberrant secretion of neurohormones, primarily the neuropeptide APGWamide, which regulates male sexual differentiation in molluscs (Oberdorster and McClellan-Green 2002). However, the body of literature is not large enough to allow full evaluation of these additional or alternative pathways.

For many years, the mechanism of TBT-induced imposex was dominated by the steroid hypothesis. This was primarily due to researchers measuring high levels of free testosterone in tissues of impacted molluscs. The link between free testosterone and penis formation was made and supported by numerous publications (e.g., Oehlmann et al. 2007). Despite extensive experimental efforts, treatment with either testosterone or fadrozole (a potent aromatase inhibitor) did not replicate this condition (e.g., Iguchi and Katsu 2008). Although it is well established that TBT does affect many CYPs (including those involved in vertebrate steroidogenesis) and other metabolizing pathways (e.g., esterification), these effects alone do not constitute evidence of a physiological role for these steroids in molluscs. In fact,

both the origin and physiological role of sex steroids in molluscs are still controversial (for reviews see Scott 2012, 2013). Current data suggest that they are likely to be accumulated from the environment. Gooding and Leblanc (2001) provided the first evidence on the ability of molluscan species to take up steroids from water, which was further supported by additional studies (e.g., Janer et al. 2004). More recently, a comprehensive evaluation for steroid uptake by both gastropod snails and bivalve molluscs (Giusti 2013; Giusti et al. 2013a; Schwarz 2015; Schwarz et al. 2017a, b) revealed that this process is very rapid, does not seem to have a saturation limit and is particularly strong for testosterone. Following steroid uptake from the environment, molluscs appear to esterify them (Giusti and Joaquim-Justo 2013) and store them as fatty acid esters through the action of acyl-CoA: testosterone acyl transferase (ATAT) (Janer et al. 2004: Gooding and Leblanc 2001). The retention of steroids as fatty acid esters appears to persist as there is little to no depuration, particularly of estradiol, testosterone, and progesterone from either snails or bivalves placed in clean water for up to 10 days (Schwarz 2015; Schwarz et al. 2017a, b).

Inhibition of either CYPs or phase II metabolism by TBT inevitably will reduce steroid clearance and metabolism, leading to an increase in free steroids. It has been suggested that TBT acts by reducing the retention of testosterone as fatty acid-esters, thus increasing the levels of free testosterone. This may play a role in the development of male sexual organs in females (LeBlanc et al. 2005). However, LeBlanc et al. (2005) added that two assumptions must be met before this putative causative relationship between TBT, testosterone, and imposex can be accepted. First, it must be accepted that testosterone is a male sex-differentiating hormone in molluscs and second, TBT specifically targets some component of the testosterone regulatory machinery causing the aberrant accumulation of this hormone in the snails. None of these has been proven to date. The lack of a nuclear AR or AR-like homologues in the genomes of molluscan species studied to date supports the lack of a physiological role for androgens in these species (Kaur et al. 2015; Vogeler et al. 2014).

4.2.2 Fish

The MIE for reproductive and metabolic impairment in fish is also expected to occur via the RXR and/or PPAR receptors. Lima et al. (2015) exposed zebrafish to only one dose each of TBT, cis-9-RA, and all-trans-retinoic acid in the diet. TBT at this one dose (2.4 μg/g in diet) affected fish weight, fecundity, and sex ratio; however, the natural RXR ligand (cis-9-RA) at 5 μg/g in diet did not. Zhang et al. (2013b) reported significant activation of RXRα in male rockfish (*Sebastiscus marmoratus*) brain when exposed to TBT at 2.4, 24, and 244 ng TBT/L. Female rockfish exhibited the opposite pattern with significant reductions in expression at the two highest doses. No differences in expression of $PPAR_{\gamma}$ were found for males or females at any dose. Several studies reported TBT-induced effects on adipogenesis via RXR/PPAR activation (Meador et al. 2011; Tingaud-Sequeira et al. 2011; Ouadah-Boussouf and Babin 2016).

How exactly the RXR/PPAR activation affects reproduction in fish however is poorly understood. Diverse evidence point to the existence of RXR/PPAR and ER cross-talk. It has been shown however that PPAR and RXR, or their heterodimer, can bind directly to estrogen responsive elements (EREs) in the gene promoters (Nunez et al. 1997). Other studies suggest that RXR/PPAR activation directly affects CYP expression, including aromatase (Cheshenko et al. 2008). A PPAR/RXR responsive element was predicted in the zebrafish CYP19β promoter (Kazeto et al. 2001) whilst a RAR binding region was identified in the tilapia CYP19 promoter (Chang et al. 2005). This was further supported by in vitro studies with mammalian cell lines where TBT binding to RXR and PPAR leads to modulations of CYP19 expression (Nakanishi et al. 2005, 2006).

4.2.3 Mammals

As described above, Kanayama et al. (2005) presented some of the first evidence of the implication of the RXRα and $PPAR_{\Upsilon}$ pathways in TBT-mediated endocrine effects in mammals, and also proposed that this pathway is the likely route for the low-dose imposex response in gastropods. Additional studies (Nakayama et al. 2005; Grün et al. 2006; Le Maire et al. 2009) provided further data showing that TBT exerts its biological effects via transcriptional regulation of gene expression through activation of these receptors, implicating this pathway in the adipogenic effects of TBT. Mammalian aromatase gene expression is also regulated through the RXRα/$PPAR_{\Upsilon}$ pathway by various ligands, including TBT. However, the direction of the regulation in response to TBT appears to be dose- and tissue-specific, potentially because aromatase is regulated through tissue-specific promoters (Simpson et al. 1993).

4.2.4 Summary

Retinoic acid binds to both RAR and RXR regulating the transcription of 500 genes involved in a large array of biological processes (Blomhoff and Blomhoff 2006). RA has long been recognized as a morphogen, important for axial patterning and organ formation in developing vertebrates. An adverse outcome pathway for neural tube and axial defects modulated by retinoic acid in vertebrates (including man) has been proposed (Tonk et al. 2015). This analysis was based on data from rat whole embryo culture, embryonic stem cells and the zebrafish embryotoxicity test, and identified certain conserved pathways on RA signaling between mammals and fish. Adverse effects in vertebrates included craniofacial and limb malformations/defects, which suggests that the TBT-induced developmental abnormalities in mollusks, such as shell abnormalities (Alzieu et al. 1986), and imposex are different expressions of disruption of the same pathway. Analysis of genomic data revealed that the important morphogenic role of RA does not only extend to invertebrate

chordates (tunicates and cephalochordates), but also to other invertebrate groups, such as hemichordates and sea urchins (Campo-Paysaa et al. 2008).

Altogether evidence suggests that most of the endocrine effects of TBT have their origin in RXR and RXR/PPAR activation. An important determinant of the severity and magnitude of responses in different species is the structure of the ligand binding domain, as studies have demonstrated the evolutionary plasticity of this domain, whilst the function of RAR, RXR, and PPAR appears to be largely conserved.

5 Toxic Effects Plausibly Mediated by Endocrine Disruption

5.1 The OECD Conceptual Framework for Endocrine Disruptor Testing and Assessment

As previously mentioned, few studies were performed according to standard test guidelines that correspond to the OECD Conceptual Framework for Endocrine Disruptor Testing and Assessment (CFEDTA; see Table 1 of the Annexes) (OECD 2012a, b). Non-standard studies do not necessarily fit within the different levels of this framework. Hereafter, the studies with TBT are therefore organized according to the test design, including the exposure duration and portion of the life-cycle exposed, and to the type of biological responses. As EC_x were not always provided or could not be deduced from the study, endpoints are mainly reported as LOEC and/or NOEC.

Standard ecotoxicology data characterizing acute toxicity endpoints were not reviewed in any great detail for this review because overt toxicity did not impact the evaluation of endpoints relevant to endocrine disruption. Acute toxicity for sensitive species was generally one to two orders of magnitude greater than LOECs for the relevant chronic apical and endocrine disruption endpoints.

5.2 Non-test Approach: Summary of Toxicological Information

The level 1 of the OECD CFEDTA corresponds to non-test information that can be used to define the general toxicological profile of a chemical with respect to its endocrine disruption properties (OECD 2012a). QSAR analyses have been conducted in some organisms (e.g., green algae; Neuwoehner et al. 2008). However, they only provide patchy information and do not appropriately cover taxa of interest such as molluscs or fish. Therefore, in the context of the present review, TBT has been evaluated in the US EPA's ToxCast™ program (http://epa.gov/ncct/toxcast/). It was classified as being a "promiscuous" chemical because it exhibited

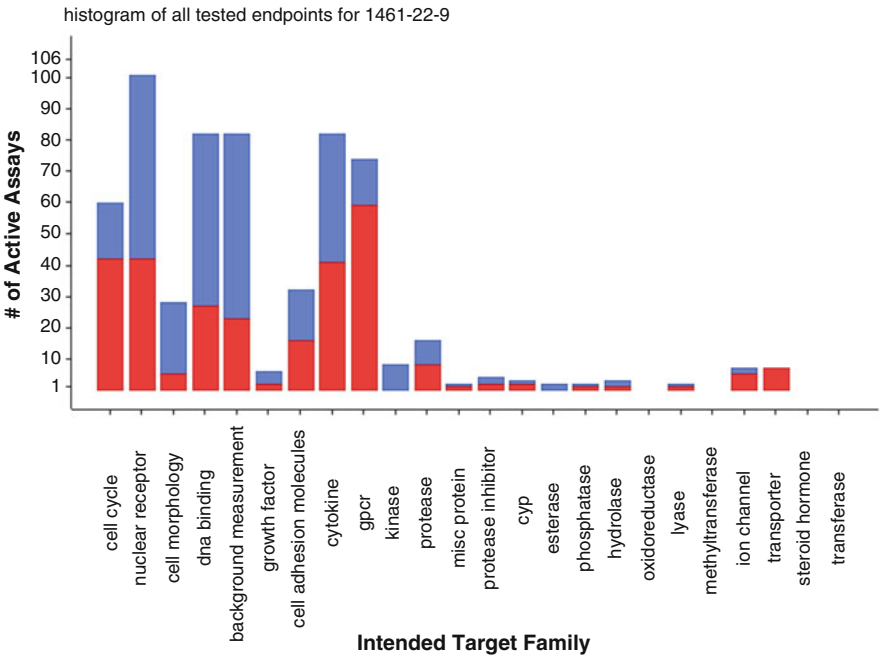


Fig. 2 Result of the ToxCast™ analysis for TBT (*red* = active assays and *blue* = inactive assays) (Downloaded March 2016)

activity in approximately 285 assays across 20 target families (Fig. 2). This type of profile suggests that TBT will act across a number of toxic pathways potentially including endocrine and non-endocrine molecular initiating events.

5.3 Invertebrates

5.3.1 In Vitro Assays

In molluscs, few studies have reported the use of in vitro assays for identifying initial endocrine mechanisms (i.e., MIE) due to TBT exposure. These studies employed transactivation assays using mammalian cells transfected with RXR from *Thais clavigera* (Urishitani et al. 2013) and *Lymnaea stagnalis* (Boulahtouf et al. 2015). The results demonstrated that TBT binds the LBD of RXR in these species, and its affinity is equivalent to that of 9-cis-RA, the natural ligand of RXR.

5.3.2 Physiological Responses

In the reproductive tissues of female dog whelk *N. lapillus*, RXR gene transcription is increased in the penis-forming area associated with the formation of a penis

and/or vas deferens (Lima et al. 2011). In this species, 9-cis-RA is as potent as TBT in inducing imposex, indicating that TBT toxicity in gastropods is mediated through the modulation of the RXR signaling pathways (Nishikawa et al. 2004; Castro et al. 2007). It should be noted that imposex is also induced by rosiglitazone, a PPARγ agonist, suggesting that the heterodimer of RXR-PPAR is a critical pathway for this phenomenon, at least in *N. lapillus* (Pascoal et al. 2013). This study also suggested that other transcription factors such as LXR and lipophilic orphan receptors are involved in the TBT toxicity pathway.

Interestingly, TBT appears to affect the endocrine system of insects as well (Hahn and Schulz 2002). In an in vivo study on *Chironomus riparius*, an environmentally relevant concentration of TBT (1 ng/L) showed effects on many endocrine-related genes, including up-regulation of the ecdysone receptor, the ultraspiracle gene (the orthologue of RXR in insects), the estrogen-related receptor, and the E74 early ecdysone inducible gene, whilst the vitellogenin (Vtg) gene remained unaffected (Morales et al. 2013). The same study showed genotoxic effects of TBT in insects by means of the comet assay.

5.3.3 Organismal Effects

A wide variety of organ responses are known in molluscs exposed to low concentrations of TBT. These include penis development in female snails, abnormal testis histopathology, and sperm alteration (count, motility, abnormality). These responses occur in the 1–10 ng TBT/L range for aqueous concentrations in the environment (e.g., Horiguchi et al. 1994; Leung et al. 2006) and 10 to 100 ng TBT/g wet weight (ww) whole-body tissue (Meador 2011) in affected gastropods. Female prosobranch molluscs exposed in the laboratory can develop imposex with LOECs of 1 to 83 ng/L (Gooding et al. 2003; Abidli et al. 2012, 2013).

In gastropods, adverse effects resulting from in vivo exposure to TBT mainly concern reproductive impairment associated with alterations of the sexual organs in females, including the staged development of penis and vas deferens known as imposex. The Vas Deferens Sequence Index (VDSI) has been developed to characterize the extent of imposex in *N. lapillus* and *T. clavigera* (e.g., Gibbs et al. 1987; Blackmore 2000; Leung et al. 2006). VDSI has seven stages, with stage 0 indicating no imposex and stage 6 indicating female sterilization. Several studies indicate that the imposex threshold concentration lies at 1 ng TBT/L, with increasing sterilization as concentrations increase (e.g., Gibbs et al. 1988). Gibbs (1996) found that juvenile female *Ocenebra erinacea* exposed to 7.3 ng TBT/L developed a longitudinal split of the oviduct wall. Adult *O. erinacea* collected from TBT-contaminated sites with advanced imposex exhibited the same lesions and through laboratory spawning experiments were found to be sterile (no capsules produced). Significant reductions in gastropod reproduction were generally found at levels slightly higher than those that induced imposex, with effective concentrations ranging from 12 to 1000 ng TBT/L (Duft et al. 2007; Leung et al. 2007; Giusti et al. 2013a, b). Other invertebrate species are known to exhibit reproductive effects across a wide

aqueous concentration range of 10 to 2225 ng TBT/L (Oberdorster et al. 1998; Ohji et al. 2003a, b; Huang et al. 2010).

Developmental toxicity of TBT was also investigated, using a 21-day embryo test with the freshwater snail *Lymnaea stagnalis*. TBT (added as TBTCl) had NOEC values of 30 ng TBT/L and 100 ng TBT/L for the mean hatching time and hatching success, respectively (Bandow and Weltje 2012). These values compare well with the NOEC of 105 ng TBT/L obtained for the fecundity of adult *L. stagnalis* exposed to TBT hydride for 21 days (Giusti et al. 2013a, b).

5.3.4 Life-Cycle Studies and Population-Level Responses

Limited full life-cycle or multigenerational laboratory studies using TBT have been conducted with invertebrates other than molluscs. The calanoid copepods *Pseudodiaptomus marinus* and *Schmackeria poplesia* exhibited similar sensitivities, with LOECs of 6 and 20 ng TBT/L, respectively (Huang et al. 2006, 2010). The amphipod *Caprella danilevskii* exhibited similar sensitivity to the copepods with a LOEC of 10 ng TBT/L (Ohji et al. 2003a, b). *Daphnia magna* was thought to be much less sensitive to TBT, with a LOEC of 2225 ng TBT/L in a two-generation study (Oberdorster et al. 1998). However, a recent study reported reduced brood size, total offspring, neonate volume, and neonate length at 88 ng TBT/L, with many of these effects being observed in the F1 and F2 generations (Jordão et al. 2015). Altered lipid homeostasis was suggested to be responsible for the abnormalities in reproduction.

For gastropods exposed to TBT, life-cycle and population studies are mainly represented by long-term laboratory exposures (>1 year) or field monitoring. In gastropods, a large number of studies reported decline to extinction for populations with increasing proportions of imposexed females (Gibbs 1996, 2009). Several studies indicate that TBT in the marine environment can impact populations of Caenogastropod snails through female sterilization associated with imposex (Spence et al. 1990; Bailey et al. 1995; Harding et al. 1997). These population-level responses were associated with water concentrations in the range 1–10 ng TBT/L, which is consistent with molecular studies characterizing the affinity of TBT for the RXR-PPAR receptor, in addition to other known ligands. It is important to note that there is not always a linear relationship between imposex development, as assessed through the VDSI, and female sterility (Barroso et al. 2002). On the other hand, female gametogenic activity occurs with no apparent differences in imposex-affected and imposex-free populations (Avaca et al. 2015). This may have important implications for population recovery.

Shell development in molluscs may be as sensitive as imposex and reproduction with a number of species exhibiting shell growth and development effects in aqueous concentrations ranging from 8 to 1000 ng TBT/L (Leung et al. 2006, 2007; Giusti et al. 2013b). Shell abnormalities in oysters were reported as early as the beginning of the 1980s (Alzieu et al. 1986), showing that oyster populations have also been impacted by TBT. For example, a correlation was found between

rock oyster (*Saccostrea glomerata*) population density and the discontinued use of TBT in estuaries with high densities of boat moorage (Birch et al. 2014). This conclusion is also supported by other studies that reported declining tissue concentrations in molluscs from this area over the same time period (Batley et al. 1992; Lewis et al. 2010).

5.3.5 Reversibility of the Effects

The degree of imposex reversibility in molluscs depends on the species. Once TBT exposure stops, female penis length declines slowly in *Nassarius reticulatus* (Bryan et al. 1993), and more quickly in female *Ilyanassa obsoleta* (Smith 1981). Contrary to this, imposex in *N. lapillus* is largely irreversible (Bryan et al. 1987). Population recovery can therefore be slow, especially for species that are long-lived and/or for which recruitment is limited, such as *N. lapillus* (Matthiessen and Gibbs 1998; Oehlmann et al. 2007; OECD 2010) and *N. reticulatus* (Couceiro et al. 2009). Nevertheless, there appears to be a widespread amelioration worldwide (e.g., Canada, US EPA 2003; Hong-Kong, Leung et al. 2006; Spain, Couceiro et al. 2009; England and Wales, Nicolaus and Barry 2015) as populations of snails have recovered significantly after the reduction in use of TBT as an antifoulant (Birchenough et al. 2002; Bray et al. 2012; Nicolaus and Barry 2015).

5.4 Fish

5.4.1 In Vitro Assays

Organotins, including TBT, are well-known inhibitors of the hepatic microsomal CYP450 systems in a variety of fish species (review by Fent and Hunn 1996). Protein transcription, enzyme activity, and reductases are all affected. The isoform CYP450 1A1 appears to be particularly sensitive to TBT exposure. Other CYP450 isoforms, including those with testosterone hydroxylase activity, are also inhibited, albeit at high concentrations. In general, CYP450 inhibition occurs at levels that are close to cytotoxicity and as such it is difficult to establish whether the inhibitions observed constitute an additional mechanism of toxicity, or they are indeed an effect of general toxicity affecting a variety of sulfhydryl-containing proteins. It has since been established that not only phase I but also phase II metabolism enzymes are affected by TBT (Morcillo et al. 2004). It is interesting to note that in this study, glucuronidation of testosterone but not estradiol was inhibited by incubating fish liver microsomes with TBT at concentrations as low as 5 μM.

The general inhibitory effect of moderate to high doses of TBT on hormonal and biotransformation pathways has also been confirmed in salmon hepatocytes using gene expression patterns (Vtg, ER, AR) and CYP-mediated enzyme activities as endpoints (Mortensen and Arukwe 2009). These consistent decreases in cellular

responses over time and with increasing TBT concentrations suggest a possible inhibitory effect of TBT on transcription. The effect of TBT on other transcription factors such as RXR and PPAR in fish cell systems is surprisingly under-studied. Nevertheless, TBT inhibited plaice (*Pleuronectes platessa*) $PPAR_{\alpha}$ and $PPAR_{\beta}$ at 1 nM in transfection assays although it had no effect on $PPAR_{\gamma}$ (Colliar et al. 2011).

5.4.2 Physiological Responses

Several studies have examined various physiological responses in fish exposed to TBT. A series of studies have been published on TBT-induced effects on rockfish (*Sebastiscus marmoratus*) gonadal development. In the first study, Zhang et al. (2007) brought wild female fish into captivity for experimentation. Ovarian testosterone levels of fish exposed to 10 ng TBT/L significantly increased, whereas levels in ovaries exposed to 100 ng/L did not significantly change compared to the control. Exposure to 10, 100 ng/L TBT resulted in significantly decreased 17β-estradiol levels in the ovaries. No masculinization was observed but the exposure period, limited to 50 days, might have been too short or the sexual differentiation stage as female was too advanced. Histological examination reportedly showed developmental suppression and atresia of ovarian follicles. However, these results may be unreliable as they included incorrect criteria to judge the follicles development stage [e.g., follicles labeled vitellogenic were not vitellogenic, and the results of Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay staining for apoptosis were not convincing; J. Wolf, pers. com. 2016]. Likewise, there was no mention of methods used to minimize observational histopathological bias.

In a second experiment (Zhang et al. 2009a, b), fish were exposed to nominal concentrations of 2.44, 24.4, and 244 ng TBT/L for 48 days. There was strong evidence of inhibition of testicular development, interstitial fibrosis and increased testicular lipid but no NOEC (only a LOEC) was established. Histopathological testicular changes were observed that may represent a stress response as opposed to specific endocrine or toxicological activity (Zhang et al. 2009a). Inhibition of thyroidal status related to depression of testicular development was also studied at TBT concentrations of 2.44, 24.4, and 244 ng TBT/L for 50 days. The NOEC was 2.44 ng TBT/L based on histopathology (testis: decreased spermatozoa, pyknosis, interstitial fibrosis; thyroid: decreased colloid). There was a possible effect of decreased spermatozoa at 244 ng TBT/L, and damage to the thyroid gland and a decrease in production of thyroid hormones were observed (T4 in serum significantly correlated with GSI) (Zhang et al. 2009b).

In a third study (Zhang et al. 2011), TBT-induced RXR_{α} expression in embryos at 0.1 and 1 ng/L, an effect thought to be responsible for the induction of increasing apoptotic cells. TBT was also shown to induce ectopic lipid accumulation in ovarian interstitial cells (consistent with PPAR activation) and decreased testosterone esterification in the ovaries leading to increased free testosterone (NOEC = 1 ng/L).

In a final study (Zhang et al. 2013a, b), 30 male and 30 female fish per group were exposed to nominal concentrations of TBT of 1, 10, and 100 ng/L. CYP19b expression in the male fish significantly increased ($p = 0.026$, 0.04, and 0.02, respectively) after exposure to TBT, the highest elevation being 3.12-fold in the 10 ng/L group. In the female fish, the CYP19b expression increased slightly in the 10 and 100 ng/L groups, but this was not statistically significant ($p = 0.078$ and 0.234, respectively). Testosterone and estradiol levels were unchanged in males but testosterone increased and estradiol decreased in females. ER expression was affected in males (up-regulated at low concentrations; no difference at 100 ng/L) but not in females. RXR_{α} expression increased in males but decreased in females.

TBT is a complex endocrine disrupter in zebrafish (*Danio rerio*). McGinnis and Crivello (2011) injected TBT at 1–5 mg/kg intraperitoneally into fish, which directly inhibited ER-regulated processes by acting as a non-competitive inhibitor. TBT did not inhibit AR-regulated processes, but decreased acyl-transferases and sulfation of testosterone in the liver. TBT had an androgenizing effect in the brain but a feminizing effect in the liver and gonads. Rapid metabolization of TBT to di- and monobutyltin also occurred in the liver, resulting in complex and non-elucidated interactions with steroid pathways (McGinnis et al. 2012).

In the brown trout (*Salmo trutta fario*), TBT exposure showed a decreasing trend of ovarian CYP19 expression, but not a potent effect at 1000 ng/L (Pereira et al. 2011a, b), suggesting that TBT interferes with the steroidogenic pathway at a transcriptional level. The in vivo down-regulation of IGF2 in the pre-vitellogenic ovaries might indicate that TBT interferes with factors that are absent in the ex vivo gonad cultures (Pereira et al. 2011a, b). TBT did not affect testosterone or estradiol concentrations, further supporting previous evidence that the CYP19 modulating effects of this chemical are not mediated through direct inhibition of CYP19 activity (Pereira et al. 2011a, b). Aromatase expression in the brain, reproductive behavior, and secondary sexual characteristics were studied by Tian et al. (2015) in guppies (*Poecilia reticulata*). TBTCl treatment inhibited gene expression of CYP19A and CYP19B in brain of males, which led to altered reproductive behavior with a LOEC of 4.45 ng TBT/L.

A series of studies with juvenile Atlantic salmon (*Salmo salar*) that were force-fed TBT alone and in combination with forskolin, reported a number of affected gene expression patterns, including CYP3a, CYP11b, CYP19a, SF-1, glucocorticoid receptor, ERα PXR, PPARs, glutathione *S*-transferase (GST), ACOX 1, IL-b, TNFa, IFNγ, IFNα, Mx3, IGF-1, IL-10, and TGFb (Kortner et al. 2010; Pavlikova et al. 2010, Pavlikova and Arukwe 2011). Forskolin activates the enzyme adenylyl cyclase and increases intracellular levels of cAMP, an important second messenger necessary for the proper biological response of cells to hormones and other extracellular signals. Since most effects observed after TBT exposure were modulated by forskolin exposure, these studies suggested that TBT may exert its endocrine, biotransformation and lipid peroxidation effects via the cAMP/PKA second messenger system.

5.4.3 Organismal Effects

In contrast to the extensive literature dealing with the adverse impacts of TBT in mammalian and molluscan species, relatively few studies have addressed higher-level effects of TBT on fish. Bentivegna and Piatkowski (1998) studied embryotoxicity of TBT in medaka *Oryzias latipes* exposed to nominal concentrations of TBT acetate (TBTA). Results showed that 415 and 4150 ng/L produced 100% lethality in all age groups, while 41.5 ng/L produced no acute lethality in 3- and 5-day embryos, and between 16 and 33% lethality in 0-day embryos. Subchronic endpoints showed that toxicity was concentration-related and that embryos exposed on day 0 were more sensitive than those exposed on days 3 and 5. LOECs for hatching success were 10,440 ng/L for day 0 and 41,500 ng/L for days 3 and 5. LOECs for the combined effects of hatching success and gross abnormalities were 10,440 ng/L for day 0 and 20,590 ng/L for days 3 and 5. Although no endocrine-sensitive endpoints were measured, there was some evidence of reduced CYP450 induction.

It has been reported that TBT can alter the sex ratio towards males in zebrafish (McAllister and Kime 2003; Santos et al. 2006) and Japanese flounder (*Paralichthys olivaceus*) (Shimasaki et al. 2003). Genetic female flounder exhibited an increased rate of sex reversal when fed TBT in their diet. The proportion of males significantly increased to 25.7% in the 0.1 μg/g group and to 31.1% in the 1.0 μg/g group compared with the control (2.2%). Histological observations showed that, in the TBT-treated groups, normal females had typical ovaries and assumed sex-reversed males had typical testes without intersex (Shimasaki et al. 2003). Zebrafish exposed from 0 to 70 days post-fertilization (dpf) to 0.1 ng/L of TBT showed a male-biased population and produced a high incidence of sperm lacking flagella. At 1 ng/L, the motility of sperm was significantly lower than that of control fish, while at 10 ng/L, all sperm lacked flagella, and at 100 ng/L, milt volume increased. Male sex ratio shifts were similar after exposure from 0 to 70 dpf and 0 to 30 dpf. Equally important, 100 ng/L resulted in 65% males after exposure from 30 to 60 days emphasizing the point that timing of the exposure is very important. Effects on sperm motility and morphology and on milt volume were less pronounced after 30 days than after 70 days of exposure (McAllister and Kime 2003). From this study, the NOEC and LOEC values were 0.01 and 0.1 ng/L, based on nominal exposure concentrations. In a recent study, Lima et al. (2015) exposed zebrafish larvae from 5 dpf up to 120 dpf to 1466 ng TBT/g diet. Animals were fed this diet three times per day. Females were significantly smaller and weighed less, while no change in male total length or weight was observed. Gonad weight in males was significantly heavier but no change was observed in females. There was a 62% decrease in fecundity but no changes in egg viability or hatchability. Overall, sex ratios shifted towards females in contrast to other studies reported above. This could be a strain difference or a concentration effect. The expression of gonadal aromatase was unaffected but in female brain, TBT down-regulated CYP19a1b mRNA. There was also a brain-specific down-regulation of $PPAR_{\gamma}$ in both males and females. TBT effects in zebrafish may involve

modulation of $PPAR_{\gamma}$/RXR and brain aromatase based on this study. A LOEC for all endpoints of 1466 ng TBT/g diet was reported, based on measured concentrations.

Reduced sperm counts were observed in guppies (*P. reticulata*) following exposure to 11.2 or 22.3 ng TBT/L for 21 days, with possible effect on Sertoli cell function. This potentially occurred via apoptosis, which could block the nutritional activity of Sertoli cells on maturing spermatids and thereby arrest the release of gametes. TBT exposure for 21 days decreased sperm counts in guppies by 40–75% but flagellar length was unaffected. However, this exposure involved adult fish and effects on sperm were short-term. Sperm counts declined approximately 62–69% but there was no change in testes size or sperm length (Haubruge et al. 2000). In a histological evaluation of TBT's toxicity on spermatogenesis, Mochida et al. (2007) exposed mummichog (*Fundulus heteroclitus*) to mean measured TBT concentrations of 0 (control and solvent control), 0.20, 0.54, 1.0, 1.7, 1.9, and 2.8 μg/L. In this study, there was a relatively small group size and no mention of the methods used to minimize sampling or observational bias. Some of the histopathological changes were difficult to confirm at low magnification, and there were several low quality figure images (changes could also be autolysis). However, damage to epithelial cells of seminal ducts, and slight decrease in spermatozoa numbers were reported with a NOEC-based on histopathology of 1.7 μg TBT/L. TBT can also affect sexual behavior and reproduction in medaka (*Oryzias latipes*) (Nakayama et al. 2004).

Growth effects in fish were also found at very low tissue concentrations. Shimasaki et al. (2003) reported a statistically significant decrease in body weight and length at 18 ng/g body wet weight; however, Meador et al. (2011) found significant increases at essentially the same whole-body concentration in Chinook salmon. Increased growth and lipid content data reported by Meador et al. (2011) are consistent with the mammalian response data characterizing TBT as an obesogen (Grün et al. 2006).

Several studies examined effects of maternally transferred TBT using different routes of exposure including dietary (Nakayama et al. 2005; Shimasaki et al. 2006) and injection (Hano et al. 2007). Adverse effects were noted when TBT concentrations were approximately 5–160 ng/g egg wet weight. These studies support the conclusion that fish embryos are very sensitive to TBT and indicate that maternal transfer is an important route of exposure.

5.4.4 Life-Cycle Studies

Mochida et al. (2010) exposed mummichog *Fundulus heteroclitus* in a fish full-life-cycle assay from the embryo stage until the hatch of the F1 generation at nominal TBTO concentrations of 0 (control and solvent control), 0.13, 0.25, 0.50, and 1.0 μg/L. The mean measured equivalent TBT ion concentrations corresponding to these exposure groups were 0, 0.054 ± 0.005, 0.12 ± 0.02, 0.26 ± 0.02 and 0.37 ± 0.05 μg/L, respectively. In a second experiment, nominal concentrations of

0.13, 0.50 and 2.0 μg TBTO/L were measured as 0.034 ± 0.00, 0.21 ± 0.07 and 0.81 ± 0.02 μg/L, respectively as the equivalent TBT ion. In the F0 generation, TBT exposure resulted in a male-biased sex ratio, an increase in the frequency of the appearance of apoptotic cells in the testis in maturing stages, and a decrease in fecundity. In the F1 generation, time to hatch and hatchability were all markedly affected. Exposure did not affect the proliferation of the germ cells in the testes; however, a significant increase in the number of apoptotic cells in the testes was induced. LOECs for sex differentiation (towards males), reduced spermatogenesis (increased apoptotic cells), and reduced hatching were 0.26, 0.06, and 0.05 μg/L, respectively.

5.5 *Amphibians*

5.5.1 In Vitro Assays

Few studies have been identified that examined the effects of TBT on amphibians in vitro. Using transiently transfected Cos7 cells, Grün et al. (2006) demonstrated that exposure to 60 nM TBT (presumably TBTCl, although compound, purity and source were not specified) activated RXRα and RXRϒ from the amphibian *Xenopus laevis*. Choi et al. (2007) found that TBT inhibited *Rana dybowskii* oocyte maturation in vitro (ED50: 0.6 and 0.7 μM).

5.5.2 Physiological Responses

Mengeling et al. (2016) found that 1 nM TBT (equiv. 290 ng/L) greatly potentiated the effect of T3 on thyroid hormone-induced morphological changes in *X. laevis* but showed both gene and tissue specificity in this capacity. The data also demonstrated that as an RXR agonist, TBT can disrupt TH signaling with outcomes identical to those caused by synthetic RXR-selective ligands and suggested that TBT is not activating a permissive NR-RXR heterodimer such as $PPAR_{\gamma}$ to achieve this effect.

5.5.3 Organismal Effects

Amphibian embryo development and tadpole metamorphosis are also sensitive to TBT exposure. *Xenopus tropicalis* embryos showed developmental and survival effects when exposed to TBT at 50–400 ng/L in the frog embryo teratogenesis assay-*Xenopus* (FETAX) (Guo et al. 2010). These effects were time- and concentration-dependent, with significant mortality at each time interval (24, 36 and 48 h) and exposure concentration. The most common malformations in the embryos were abnormal eyes and skin hypo-pigmentation, with increased time of exposure; additional common malformations included enlarged proctodaeum and

narrow tails fins in tadpoles. Thyroid hormone is linked to eye development in *Xenopus* embryos. The authors suggest that the eye malformations and other malformations are linked to TBT exposure through binding to RXRs and that RXRs form heterodimers with the thyroid hormone receptors.

5.5.4 Life-Cycle Studies

Shi et al. (2014) exposed *X. laevis* to TBT in an Amphibian Metamorphosis Assay (AMA) (OECD TG 231) and a complete AMA (CAMA), which exposed *X. laevis* from Nieuwoop and Faber (NF) stage 46 to stage 66. They found TBT to have anti-thyroid activity in the AMA at TBT concentrations of 12.5–200 ng/L, based on decreased hind limb length in the absence of growth effects (body weight and snout to vent length) or overt toxicity, delayed development by one or two development stages and thyroid lesions characterized by mild increases in thyroid follicle height and/or mild increases in layers of follicular epithelium, and colloid depletion. The CAMA confirmed developmental delays based on front limb emergence and total metamorphosis time; however, these effects were seen in the presence of decreased body length and weight at metamorphosis at 10 and 100 ng/L. The CAMA also found that the intersex and sex ratio increased in favor of males with increasing concentrations of TBT. The intersex gonads had an ovarian cavity with testis-like tissue structure. Apical endpoints in *Xenopus* sp. metamorphosis and embryo eye development were affected by TBT concentrations as low as 12.5 ng/L (Shi et al. 2014). Although these endpoints are regulated by thyroid hormones, it is unclear from this report whether TBT acts directly on thyroid hormones, or indirectly through binding to RXRs.

5.6 Birds

5.6.1 In Vitro Assays

No in vitro studies of TBT effects on birds have been identified.

5.6.2 Physiological Responses and Organismal Effects

Although few studies of the effects of TBT have been conducted in birds, these have demonstrated some reproductive effects from exposure. A subchronic toxicity/reproduction study was performed in Japanese Quail (*Coturnix japonica*) fed a diet containing 0, 24, 60, and 150 mg TBTO per kg diet for 6 weeks (Coenen et al. 1992). No overt toxicity or treatment-related pathological or histological abnormalities were noted in parent birds, and there were no significant effects on egg production, serum alanine aminotransferase (ALAT), serum total thyroxine (TT4),

luteinizing hormone (LH), or retinol levels. Limited effects on hematology and no effects on serum biochemistry were found in these birds (Coenen et al. 1994). However, a significant decrease in hatchability and increase in embryo mortality were observed at the two highest doses. Serum calcium values determined throughout the reproduction period were found to be significantly reduced in female birds at all concentrations tested (Coenen et al. 1992). This same study was repeated by five laboratories in an inter-laboratory comparison test (Schlatterer et al. 1993), with the addition of one higher dose group (375 mg/kg feed). Results from this comparison were similar to those reported by Coenen et al. (1992). Dose-related decreases in egg weight, egg production, fertility, hatching success and survival of 14-day-old chicks were observed in most of the laboratories. The NOEC for egg weight and hatchability was 60 mg TBTO/kg feed. Faqi et al. (1999) conducted a follow-up study to examine whether a difference in responses could be observed between a 6-week exposure and a 13-week exposure to 150 and 375 mg TBTO/kg feed. The number of eggs laid, mean egg weight, fertility and hatchability were significantly lower and the percentage of cracked eggs was significantly higher at 375 mg/kg at weeks 6 and 13. Reduced eggshell thickness was observed at week 6. No effects were noted on hematological and clinical chemistry data obtained at weeks 6 or 13 and histological preparations of the organs showed no morphological changes. However, none of these studies in quail examined steroid hormone levels or any related genomic indicators from the HPG axis, nor were any effects on nuclear receptors investigated.

5.6.3 Life-Cycle Studies

No life-cycle studies of TBT effects have been reported for birds.

5.7 Mammals

5.7.1 In Vitro Assays

Studies using mammalian in vitro systems have examined interactions between TBT and nuclear receptors and the effects of TBT on enzymes involved in steroidogenesis. Saitoh et al. (2001a) found that TBT had relatively high binding affinity for androgen receptor (AR), with an IC50 of 7.6 μM, but no affinity for estrogen receptor α (ERα). Additional evidence for TBT's impacts on segments of the steroidogenic cascade was presented by McVey and Cooke (2003), who observed decreased 17-hydroxylase and 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in rat testis microsomes at 12 and 59.0 μM TBTCl. Treatment of human granulosa-like tumor cells for more than 48 h with 20 ng/mL TBTCl (~60 nM), significantly suppressed aromatase activity and estradiol production (Saitoh et al. 2001b). Cooke (2002) and Heidrich et al. (2001) reported that TBT

is a competitive inhibitor of human aromatase in vitro at 12 and 59 μM TBTCl and 5 and 50 μM TBTCl, respectively, with an IC50 of 6 μM calculated by Heidrich et al. (2001). Together, these studies initially suggested a possible inhibitory role for TBT in steroidogenesis as the primary means of endocrine disruption. However, Kanayama et al. (2005) found that TBT (10, 30, and 100 nM TBTCl) induced the transactivation function of RXR_{α} and $PPAR_{\Upsilon}$ at concentrations lower than those causing aromatase inhibition. The effect of TBT on RXR_{α} is as strong as that of its endogenous ligand, 9-cis-RA and because TBT enhanced protein-protein interaction between RXR_{α} and TIF2, the data suggested that TBT activates transcription via these receptors.

Because TBT induced the transactivation function of RXR_{α} and $PPAR_{\Upsilon}$ at concentrations lower than those required for inhibition of aromatase activity, Kanayama et al. (2005) proposed that this receptor-based pathway is the more likely route for low-dose effects such as imposex in gastropods. The greater likelihood of a role for RXR and PPAR over the aromatase-inhibition hypothesis in TBT-mediated toxicity was further supported by studies that reported opposing effects of TBT on aromatase (CYP19). Sharan et al. (2013) found that low doses of TBT (25, 50, and 100 nM TBTCl) increased CYP19 enzyme activity, mRNA expression, and estradiol production in MCF-7 cells and acted as an ERα agonist. Evidence for the induction of aromatase by TBT was initially provided by Nakanishi et al. (2002) at 30, 100, and 300 nM TBTCl in a human choriocarcinoma cell line. Evidence for the role of the RXR homodimer in aromatase induction in placental tissue was provided by Nakanishi et al. (2002, 2005) at 1, 10, and 100 nM TBTCl. However, the utility of these studies for risk assessment is weak due to the use of inappropriate statistical methods. The variation in results of these in vitro studies with regard to aromatase and ER activation is likely due to wide differences in concentrations of TBT and/or the sources for the cells used in each study, suggesting that TBT's effects may be dose- and tissue-specific.

Additional early evidence for interaction of TBT with RXR and PPAR via direct ligand binding was presented by Grün et al. (2006) using Cos7 cells (transformed Green Monkey kidney fibroblast cells) transfected with human, mouse, and frog (*Xenopus laevis*) nuclear receptors. These experiments showed activation of $RXR_{\alpha-\Upsilon}$ and slightly weaker activation of $PPAR_{\Upsilon}$ and $PPAR_{\delta}$ (same for $PPAR_{\beta}$) at 60 nM TBT (presumably TBTCl, although compound, purity and source were not specified). le Maire et al. (2009) showed that TBT activates all three RXR-PPAR heterodimers (α, β and ϒ) primarily through its interaction with RXR. In contrast to the interaction between TBT and RXR, the active receptor conformation between TBT and $PPAR_{\Upsilon}$ was less efficiently stabilized, making this side of the heterodimer a less efficient binding target for TBT (le Maire et al. 2009), confirming the observations of Grün et al. (2006).

Multiple lines of in vitro evidence exist demonstrating the roles of RXR and PPAR in TBT-induced adipogenesis in mammals. Histological examination of mouse 3T3-L1 preadipocyte cells treated with 100 nM TBTCl by Kanayama et al. (2005) revealed induction and promotion of adipocyte differentiation. This observation was supported by induction of the adipocyte-specific fatty acid-binding

protein (aP2) mRNA expression and triglyceride levels in a dose-dependent manner at 10, 30, and 100 nM TBTCl, and linked to the $PPAR_{\gamma}$ pathway by induction of $PPAR_{\gamma}$ mRNA. Induction of adipocyte differentiation was also demonstrated by Grün et al. (2006) using histology and aP2 mRNA expression in mouse 3T3-L1 cells dosed with 10 and 100 nM TBT (presumably TBTCl, although compound, purity and source were not specified). The aP2 promoter contains an RXR:PPAR response element, implicating this pathway in the observed changes. In vitro exposure of mouse multipotent stromal stem cells to 5 and 50 nM TBT (presumably TBTCl although exact compound and purity not specified) increased adipogenesis, cellular lipid content, and expression of adipogenic genes (Fapb4, $PPAR_{\gamma}$, LEP) and decreased mRNA levels of the adipogenesis inhibitor Pref-1 (Kirchner et al. 2010). The adipogenic effects of TBT in this study were blocked by the addition of $PPAR_{\gamma}$ antagonists, suggesting that activation of $PPAR_{\gamma}$ mediates the effect of TBT on adipogenesis. TBT also induced $PPAR_{\gamma 2}$ and FABP4 protein expression in bone marrow multipotent mesenchymal stromal cells at concentrations >50 nM TBTCl, resulting in lipid accumulation and terminal adipocyte differentiation (Yanik et al. 2011). Interestingly, Belcher et al. (2014) found that TBT-induced human $PPAR_{\gamma}$ in a Chinese Hamster Ovary (CHO) cell line at 1 nM TBTCl and higher concentrations, but exhibited an "inverted U" dose-response curve, with maximal induction at 100 nM TBTCl. The cause of the loss in functional reporter gene activity was unclear.

Further evidence for the critical role for RXR in mediating effects of TBT comes from in vitro studies of effects on thyroid hormone receptors. Using thyroid hormone-responsive HepG2 cells, Sharan et al. (2014) demonstrated that TBTCl treatment induced a dose-dependent decrease in tri-iodothyronine (T3)-induced thyroid receptor (TR) transactivation and altered the expression of TRβ and its co-regulators including SRC-1 and NCoR. Therefore, TBT acts as an antagonist to TRs and inhibits T3-mediated transcriptional activity. However, TRs can form heterodimers with other nuclear receptors, in particular with the RXR. RXR plays a role in both positive and negative gene regulation through thyroid response elements (LaFlamme et al. 2002). Given the potential for TBT to also activate RXR, and the importance of RXR in the negative transcriptional regulation of genes of the hypothalamo-pituitary axis by T3 (LaFlamme et al. 2002), this suggests that TBT's effects on the thyroid axis may involve multiple nuclear receptor pathways.

5.7.2 Physiological Responses

Grün et al. (2006) dosed 6-week-old male mice for 24 h with 0.3 mg/kg bw TBT (presumably TBTCl, although compound, purity and source were not specified) and examined expression of critical transcriptional mediators of adipogenesis such as RXR_{α}, $PPAR_{\gamma}$, C/EBP α/β/δ, and sterol regulatory element binding factor 1 (Srebf1) as well as known target genes of RXR_{α}:$PPAR_{\gamma}$ signaling from liver, epididymal adipose tissue and testis. TBT either had no effect, or weakly repressed RXR_{α} and $PPAR_{\gamma}$ transcription in liver and decreased RXR_{α}, $PPAR_{\gamma}$, C/EBPα and

-δ in adipose tissue and testis. C/EBPβ was strongly induced in liver and testis, but more weakly induced in adipose tissue. Proadipogenic transcription factor Srebf1 was also induced in adipose tissue. Fatty acid transport protein (Fatp) mRNA levels were up-regulated two- to three-fold in liver and epididymal adipose tissue but not testis by TBT. Additional up-regulation of genes associated with fatty acid synthesis was also noted and together, these gene expression data confirmed TBT as a potential adipogenic agent in vivo.

Thyroid-related effects of TBT have been reported in vivo in mammals (e.g., Decherf et al. 2010; Sharan et al. 2014). Thyrotropin-releasing hormone (TRH) production is controlled at the transcriptional level by T3 through TRs but also via RXR and $PPAR_{\Upsilon}$ (LaFlamme et al. 2002). Decherf et al. (2010) exposed Swiss wild-type mice to TBT through lactation after dams were gavaged with a single 40 mg TBTCl/kg dose, and examined effects in pups on hypothalamic expression of genes implicated in metabolism and regulated by T3. They found that TBT dose-dependently increased T3-independent transcription from the TRH promoter (i.e., transcription that is controlled through RXR), but had no effect on T3-dependent repression. However, the effect on T3-independent expression was not observed in pups whose mothers were exposed chronically to 0.5 mg/kg by gavage for 14 days. Additionally, this paper demonstrated that exposure to TBT has a two-pronged effect on transcription from the aromatase and tyrosine hydroxylase promoters—it significantly reduced T3-independent transcription but also abolished T3-dependent regulation, confirming the role of TBT as a T3 antagonist that had been reported in vitro. Although the exposure levels in this study were relatively high, the results lend important insights into the hypothalamic effects of TBT.

Thyroid system effects in mice have been demonstrated at significantly lower exposure levels. Swiss albino male mice exposed to three doses of TBTCl (0.5, 5 and 50 μg/kg/day) for 45 days showed hypothyroidal effects (Sharan et al. 2014). TBT exposure markedly decreased serum thyroid hormone levels, which correlated with down-regulation of thyroid peroxidase (TPO) and thyroglobulin (Tg) genes in the thyroid gland and augmented circulating thyroid stimulating hormone (TSH) levels and TSH receptor (TSHr) gene in the thyroid gland. In addition, Pax8, a thyroid-specific transcription factor (mRNA and protein) and sodium-iodide symporter (Slc5a5) (mRNA) were also down-regulated. Sharan et al. (2014) concluded that TBT induces hypothyroidism by suppressing transcriptional activity of thyroid-responsive genes and inhibiting T3 binding to thyroid receptors, thereby preventing recruitment of co-activators and corepressors on the promoters of target genes.

Zuo et al. (2014) exposed male KM mice for 45 or 60 days to 0.5, 5, and 50 μg/kg TBTCl orally administered by gavage once every 3 days, and examined effects on the pancreas, glucose homeostasis, and circulating steroid and thyroid hormone levels. Animals treated with TBT for 60 days exhibited elevated fasting plasma glucose levels and decreased serum insulin and glucagon. TBT treatment for 45 days resulted in a dose-dependent increase in testosterone levels and a decrease in 17β-estradiol levels in the testes and serum compared to the control. Serum T4 levels did not show significant alteration in the TBT-exposed group, while T3 levels

showed a reduction in the TBT-exposed group and severe damage of the thyroid gland was observed histologically in mice exposed to 50 μg/kg TBT. No histological damage was observed in the pancreas after TBT exposure for 45 days. However, the number of apoptotic cells in the pancreas increased significantly with dose. TBT treatment for 45 days resulted in a dose-dependent decrease in pancreatic ERα expression but not ERβ levels, and resulted in an elevation of AR expression. This study is the first to examine direct endocrine effects of TBT on the pancreas.

Kirchner et al. (2010) investigated effects of in utero exposure of mice to TBT on adipose-derived stromal stem cells (ADSCs). Pregnant dams received a single 0.1 mg/kg body weight dose of TBT (presumably TBTCl although exact compound and purity not specified) by gavage and stromal cells were isolated from white adipose tissue (WAT) of their 8-week-old pups. Cells from TBT-exposed mice showed increased adipogenic capacity and lipid accumulation, reduced osteogenic capacity, increased Fapb4 and $PPAR_{\Upsilon}$ mRNA expression, decreased adipogenesis inhibitor Pref-1 mRNA, hypomethylation of the promoter/enhancer region of the Fapb4 locus, and an increased number of preadipocytes in the cells. This study provided the first evidence that in utero exposure to TBT counteracts osteogenesis and induces preferential differentiation of ADSCs into adipocytes.

5.7.3 Organismal Effects

In mammals, in vivo exposure to TBT has been shown to cause reproductive and other apical effects, although early studies used relatively high exposure levels, at which the effects of TBT are unlikely to be through the RXR/PPAR pathway. Harazono et al. (1996) reported a higher rate of pregnancy failure in Wistar rats exposed to 12.2 and 16.3 mg TBTCl/kg body weight. In a follow-up study, female Wistar rats exposed to 8.1, 16.3, or 32.5 mg TBTCl/kg body weight (25, 50, or 100 μM/kg) on days 0 through 3 of pregnancy, or 8.1, 16.3, 32.5, or 65.1 mg/kg (25, 50, 100, or 200 μM/kg) on days 4 through 7 of pregnancy by gastric intubation, and their fetuses, exhibited significantly lower body weights at 16.3 and 32.5 mg/kg than controls (Harazono et al. 1998). Exposure to 16.3 mg/kg and higher produced a significant increase in the rate of implantation failure, and dosing at the same levels on days 4–7 of pregnancy caused a significant increase in the incidence of post-implantation loss (Harazono et al. 1998). The authors concluded that susceptibility to, and manifestation of, the antifertility effects of TBTCl vary with the gestational stage at the time of administration. Dosing of rat dams with TBT beginning on gestational day 8 by oral gavage caused a significant reduction of dam's body weight at 10 mg/kg body weight during gestation and postnatally (Cooke et al. 2008). At postnatal days 6 and 12, neonatal pup weights were reduced at this concentration. However, Cooke et al. (2008) also noted that at the lowest dose of 0.25 mg TBTCl/kg body weight, dam's body weight increased relative to controls. Similarly, Zuo et al. (2009) showed that exposure of male mice to TBTCl at 5 μg/kg body weight for 45 days resulted in an increase in body weight and hepatic steatosis accompanied by hyperinsulinemia, hyperleptinemia, and changes in several

metabolism-related hormones. The variation in body weight responses appears to reflect both life stages during exposure and dose, with exposure in utero likely predisposing the animal to increased adipose mass as it ages and high dose exposures resulting in body weight loses.

One of the first studies to examine the effects of in utero exposure to TBT on lipid homeostasis and adipogenesis was conducted by Grün et al. (2006) using pups from pregnant C57BL/6 mice, which were injected intraperitoneally daily from gestational day 12–18 with 0.05 or 0.5 mg/kg body weight TBT (presumably TBTCl, although compound, purity, and source were not specified). Histological examination demonstrated that TBT exposure caused a disorganization of hepatic and gonadal architecture in the pups at birth, and liver sections exhibited signs of steatosis. Adipose mass in 10-week-old TBT-treated males was significantly higher than in controls although no overt increases in body mass were noted.

Effects of in utero exposure to TBT on fetal gonad morphology have been reported in Sprague–Dawley rats (Kishta et al. 2007). Light microscopic evaluation found that the number of Sertoli cells and gonocytes was reduced in fetuses whose mothers were gavaged daily from days 0 to 19 or 8 to 19 of gestation with 20 mg TBTCl/kg. Likewise, large intracellular spaces between Sertoli cells and gonocytes and increased abundance of lipid droplets in the Sertoli cells were observed. Electron microscopy studies revealed abnormally dilated endoplasmic reticulum in Sertoli cells and gonocytes. In the ovaries, TBT (20 mg/kg, days 0–19; 10 mg/kg, days 8–19) reduced the number of germ cells by 44% and 46%, respectively. Kishta et al. (2007) also examined gonadal gene expression in the fetuses and found significant up-regulation of testicular genes related to stress response but no up-regulation of these genes in the ovary. In ovaries, down-regulation was noted of genes involved with signal transduction.

TBT resulted in early puberty and impaired estrous cyclicity in female mice exposed perinatally (1, 10, or 100 μg TBTCl/kg body weight/day from day 6 of pregnancy), although no effects on circulating sex steroids (E2 or T) were observed (Si et al. 2012). Reductions in body weight were also reported by Si et al. (2012). Identical exposures of pregnant mice to TBT dramatically decreased sperm counts and motility in male offspring but had limited effects on intratesticular and serum hormone levels, suggesting that altered expression of receptors rather than hormone levels may be involved (Si et al. 2013).

5.7.4 Life-Cycle Studies

A two-generation reproductive toxicity study was conducted in rats using dietary exposure to TBT to evaluate its effect on sexual development and the reproductive system (Ogata et al. 2001; Omura et al. 2001). Pregnant female rats were exposed throughout pregnancy until weaning to 5, 25, or 125 μg TBTCl/g diet [assuming adult female rats weigh ~150 g and eat 16 g of food per day (US EPA 1988), this equates to approximately 12, 60, and 300 μg/kg body weight/day]. F0 and F1 progeny were provided with the same TBTCl diet as their mothers. For males

(Omura et al. 2001), significant effects on monitored endpoints (reduced body weight, delayed eye opening, reduced testis, epididymis and ventral prostate weights, and decreased spermatid count) were observed primarily at 125 μg/g. Only minimal histological changes were observed in the testes. A dose-dependent increase in serum testosterone occurred only in the F1 rats, and serum E2 was affected only in the 125 μg/g groups of F1 and F2. As is commonly seen in high dose exposures, the data suggest that these results were primarily related to direct toxic effects of TBT rather than functioning through specific endocrine pathways. A similar conclusion was drawn from the results of the companion study on the female offspring of these rats (Ogata et al. 2001). Reproductive outcomes of dams (number and body weight of pups and percentage of live pups) and the growth of female pups (day of eye opening and body weight gain) were significantly decreased in the group exposed to 125 μg TBTCl/g diet.

Chamorro-García et al. (2013) conducted a follow-up study to the one by Kirchner et al. (2010), in which they exposed female C57BL/6J mice prior to conception and during pregnancy to 5.42, 54.2, or 542 nM TBT (presumably TBTCl although exact compound, source and purity not specified) in drinking water (equivalent to 0.53, 5.3, and 53 μg/kg/day) to determine whether prenatal exposure would affect subsequent generations (F1 = exposed in utero, F2 = exposed as germ cells, F3 = no exposure). Prenatal TBT exposure elicited striking transgenerational effects in males including increased white adipose tissue (WAT) depot weights, adipocyte size, and adipocyte number at most doses in the three generations. More modest changes were observed in females, yet most doses of TBT led to significant increases in WAT depot weight and adipocyte size in F1 and F2 animals. Effects on body weight were modest and not directionally consistent in both sexes. Quantitative PCR analysis of adipogenic markers in bone marrow-derived multipotent mesenchymal stem cells (MSC) revealed sharply increased expression of Zfp423 and Fabp4 and decreased expression of Pref-1, an inhibitor of adipocyte differentiation, in TBT males from all three generations. Results from female mice were similar, but with less pronounced changes in F2 and greater variability in F3 mice than in males. Osteogenic markers, ALP and Runx2, sharply decreased in F1 and F3 males and females but were primarily unchanged in F2. All three generations exhibited hepatic lipid accumulation and up-regulation of hepatic genes involved in lipid storage/transport, lipogenesis, and lipolysis. The Chamorro-García et al. (2013) results show that early-life exposure to TBT can have transgenerational effects on adipogenesis, at least through the F3 generation.

6 Adverse Outcome Pathway

The adverse outcome pathway (AOP) is a framework that summarizes existing information for a given biological pathway from the MIE, through various levels of biological organization (genetic, molecular, physiological, and organismal), and culminates with population-relevant results that can be used for ecological risk assessment. This framework was described by Ankley et al. (2010) and has been

utilized many times to highlight progressive linkages between receptor activation and population-relevant outcomes.

The present review provides the necessary information needed to populate the various AOP components, which are displayed in Fig. 3. It should be noted that this is a putative and most likely incomplete AOP that was drafted based on existing information and our current ability to interpret the available data. In order to obtain the complete AOP, far more work is needed to fill in the gaps between MIEs and key events (KE) across the different taxa and life stages (applicability domains); even more work is required to obtain a quantitative AOP as it is likely that different pathways are operating and their LOECs may be different between applicability domains. Since TBT can trigger more than one AOP, the potential for an operational AOP network is substantial and elucidation on whether this network is converging, diverging or independent in terms of adversity will be needed. All the information captured in Fig. 3 originates from invertebrate (primarily molluscan) studies, although certain pathways have been confirmed in other taxa. Besides the putative and incomplete nature of the AOP presented here, it is almost certain that the critical pathway appears to be initiated by the RXR and RXR/PPAR interactions, to include imposex as a key event and to result in complete reproductive failure and decline population trajectory.

The full AOP can only be completed when some basic aspects of endocrinology of invertebrate species, that are currently largely unknown, become available. The same holds true for the RXR and RXR/PPAR modulated pathways in vertebrate species, although some information has recently become available (Tonk et al. 2015). Nevertheless, it should be noted that unlike the majority of the currently available AOPs in the context of chemical perturbations, the adverse outcome of TBT exposure at population level is well established at least for molluscan species as the numerous publications on population extinctions globally testify.

7 Species Sensitivity Distribution (SSD) for Toxic Effects in Aquatic Organisms

7.1 Water Exposure SSDs

Species sensitivity distributions (SSDs) have been derived using apical, population-relevant data (reproductive outcomes, including fertilization, embryonic development, hatching, larval and juvenile growth, as well as sex ratio) mainly from long-term aqueous exposure studies matching Levels 4 and 5 in the OECD CFEDTA (see Tables 2 and 3 of the Annexes). These SSDs do not necessarily include all relevant species and endpoints and are not expected to be comprehensive. However, they have been built using robust endpoints from reliable studies, and therefore accurately reflect the sensitivity of major groups of aquatic organisms. Two SSDs have been constructed—one using LOECs (Fig. 4) and the other NOECs (Fig. 5) from chronic studies—using the ETX 2.1 software of the Netherlands National Institute for Public Health and the Environment (RIVM) (van Vlaardingen et al. 2014).

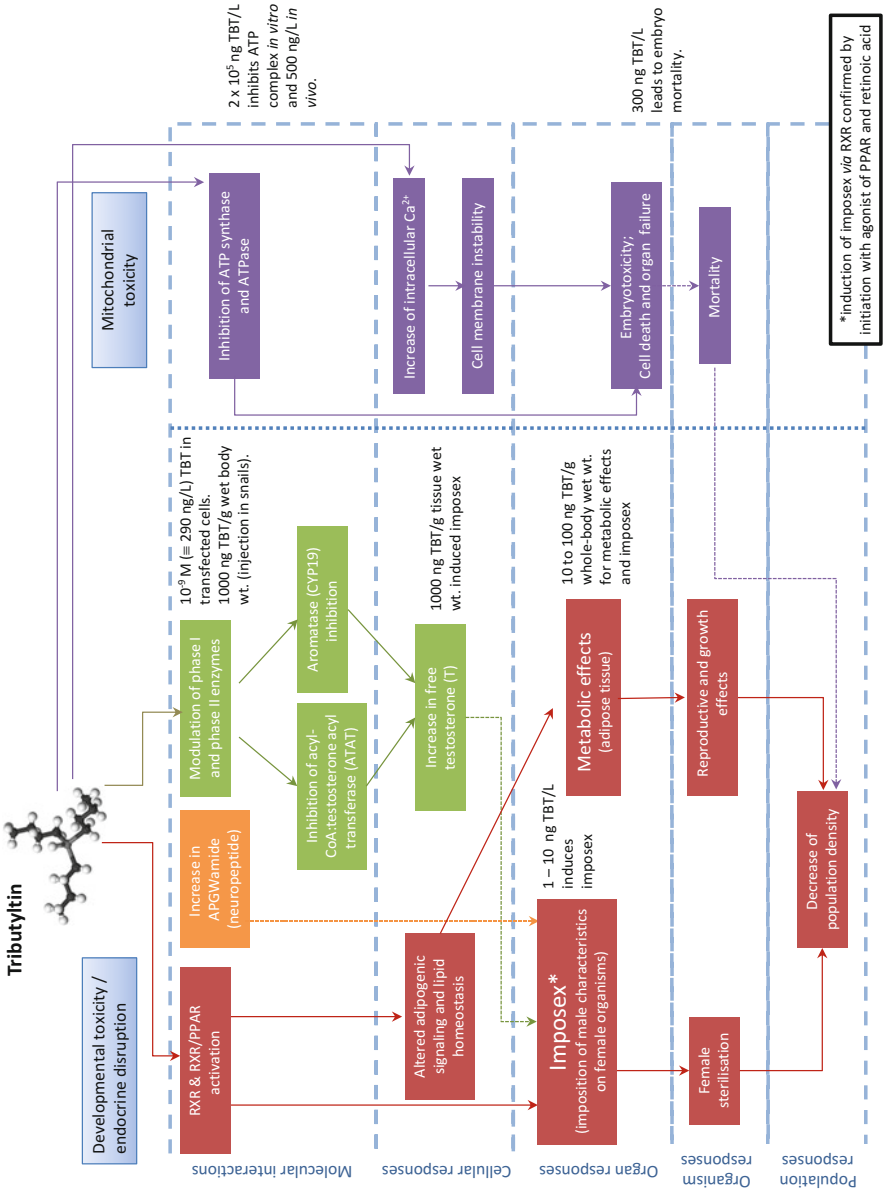
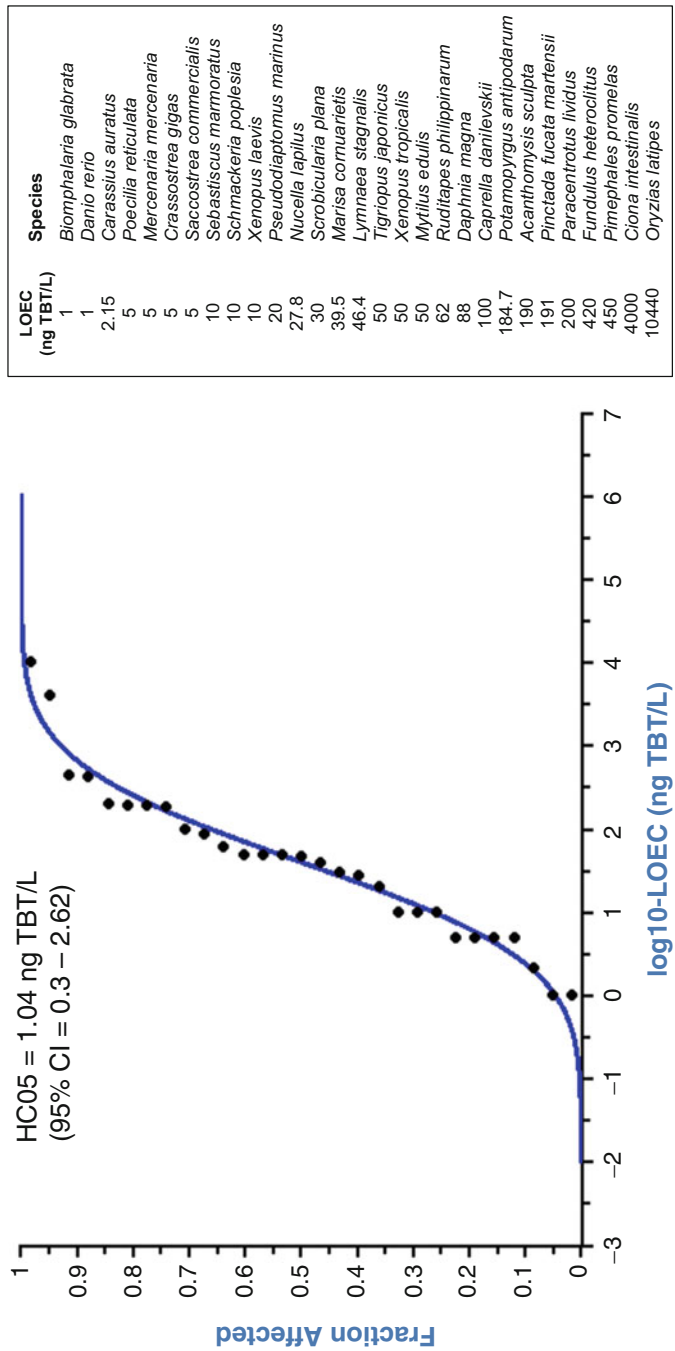
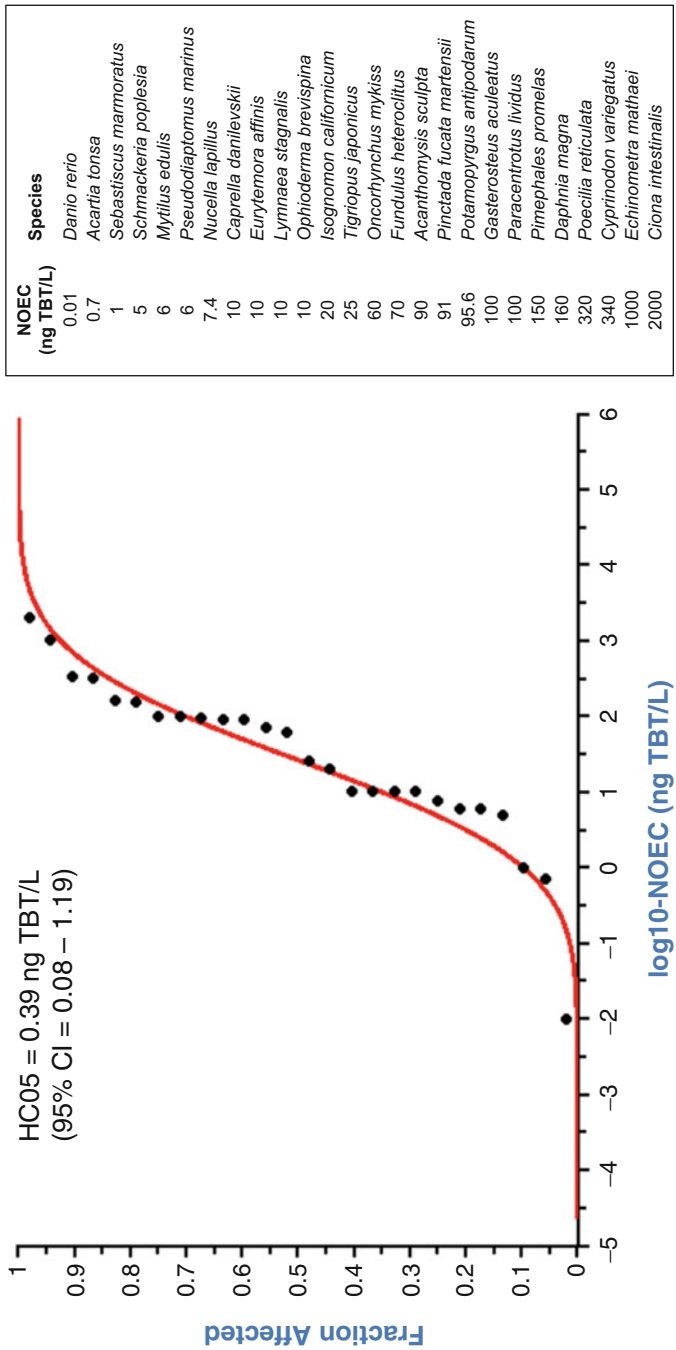


Fig. 3 Proposed adverse outcome pathway (AOP) for TBT toxicity



LOEC (ng TBT/L)	Species
1	*Biomphalaria glabrata*
1	*Danio rerio*
2.15	*Carassius auratus*
5	*Poecilia reticulata*
5	*Mercenaria mercenaria*
5	*Crassostrea gigas*
5	*Saccostrea commercialis*
10	*Sebastiscus marmoratus*
10	*Schmackeria poplesia*
10	*Xenopus laevis*
20	*Pseudodiaptomus marinus*
27.8	*Nucella lapilus*
30	*Scrobicularia plana*
39.5	*Marisa cornuarietis*
46.4	*Lymnaea stagnalis*
50	*Tigriopus japonicus*
50	*Xenopus tropicalis*
50	*Mytilus edulis*
62	*Ruditapes philippinarum*
88	*Daphnia magna*
100	*Caprella danilevskii*
184.7	*Potamopyrgus antipodarum*
190	*Acanthomysis sculpta*
191	*Pinctada fucata martensii*
200	*Paracentrotus lividus*
420	*Fundulus heteroclitus*
450	*Pimephales promelas*
4000	*Ciona intestinalis*
10440	*Oryzias latipes*

Fig. 4 Species sensitivity distribution (SSD) constructed with sublethal population-relevant LOEC values for TBT in 29 aquatic species. See Table 2 of the Annexes for the endpoint value and description for each species



NOEC (ng TBT/L)	Species
0.01	*Danio rerio*
0.7	*Acartia tonsa*
1	*Sebastiscus marmoratus*
5	*Schmackeria poplesia*
6	*Mytilus edulis*
6	*Pseudodiaptomus marinus*
7.4	*Nucella lapillus*
10	*Caprella danilevskii*
10	*Eurytemora affinis*
10	*Lymnaea stagnalis*
10	*Ophioderma brevispina*
20	*Isognomon californicum*
25	*Tigriopus japonicus*
60	*Oncorhynchus mykiss*
70	*Fundulus heteroclitus*
90	*Acanthomysis sculpta*
91	*Pinctada fucata martensii*
95.6	*Potamopyrgus antipodarum*
100	*Gasterosteus aculeatus*
100	*Paracentrotus lividus*
150	*Pimephales promelas*
160	*Daphnia magna*
320	*Poecilia reticulata*
340	*Cyprinodon variegatus*
1000	*Echinometra mathaei*
2000	*Ciona intestinalis*

Fig. 5 Species sensitivity distribution (SSD) constructed with population-relevant NOEC values for TBT in 26 aquatic species. See Table 3 of the Annexes for the endpoint value and description for each species

When several biological endpoints were available for one given species, only the lowest LOEC and/or NOEC was used, to ensure that each species appeared only once in the SSD. In some cases, the LOECs are unbounded, so the true effect concentration could be much lower. The NOEC is also sometimes significantly lower than the LOEC, depending on test design (i.e., some may be over-precautionary). By assessing the distribution of both LOECs and NOECs, these factors have been ignored for the purposes of this analysis. For both SSDs, the goodness-of-fit was acceptable (significance level: 0.01–0.1), as assessed using the Anderson-Darling, Kolmogorov-Smirnov, and Cramer von Mises tests for normality.

As a result from the NOEC-based SSD, 0.39 ng/L (95%-CI: 0.08–1.19 ng/L) was determined to be the HC5 (the predicted concentration that affects 5% of the species in the distribution), whereas the HC5 based on LOEC values was 1.04 ng/L (95%-CI: 0.3–2.62 ng/L). The NOEC-based HC5 value is close to current EU regulatory thresholds (e.g., 0.2 ng/L; EU 2005), which incorporates a safety factor and so should be treated with caution. For comparison, the USEPA ambient water quality criterion (seawater chronic) has been set to 7 ng/L (US EPA 2003).

An interesting feature of this sensitivity analysis is that some fish and other invertebrate species are more sensitive to TBT than caenogastropod molluscs, in particular some copepods (*Acartia tonsa*, *Schmackeria poplesia Pseudodiaptomus marinus*) and the fish *Danio rerio* and *Sebastiscus marmoratus*. The lowest endocrine-sensitive LOEC identified in this review was 0.1 ng TBT/L for a male-biased sex ratio change and abnormal sperm in zebrafish (McAllister and Kime 2003). Our analysis found that study convincing, well designed, and described. Aqueous concentrations of TBT in aquaria for the highest dose (100 ± 5 ng TBT/L) were verified by gas flame atomic absorption spectrophotometry while other doses were below the detection limits. Because this is the lowest value, it sets the lower limit concentration for the TBT risk assessment for fish and also for molluscs and other invertebrate species.

Such a high sensitivity of zebrafish sex ratio to TBT is consistent with the fact that TBT inhibits aromatase, thus increasing the level of free testosterone to which zebrafish appear to be extremely sensitive (Holbech et al. 2006; Örn et al. 2006; OECD 2012b). There is therefore a biological plausibility that links the molecular initiating event (aromatase inhibition) to the key event (increased levels of free testosterone) subsequently leading to an apical effect (male-biased sex ratio), which is meaningful for inferring potential impact at the population level. Such causality relationships were thoroughly described by Matthiessen and Weltje (2015) for azoles compounds, which also act as aromatase inhibitors.

Comparison of the potencies of known aromatase inhibitors, such as prochloraz, with TBT requires an in-depth analysis of the available data. However, this is somehow out of the scope of this review. Nevertheless, it appears that, with an IC_{50} around 0.2 μM (Cooke 2002), TBT is a weaker aromatase inhibitor in vitro as compared to prochloraz (IC_{50} = 0.04 μM; Vinggaard et al. 2000). In contrast, in vivo (zebrafish fish sexual development test), a dramatic shift in potencies was observed with TBT affecting sex ratio towards males at 1 ng/L (McAllister and

Kime 2003) whilst prochloraz shifted sex ratio towards males at 202 µg/L (Kinnberg et al. 2007). It is highly unlikely such a large difference stems from slightly different exposure conditions. Hence, it could be speculated that TBT exerts its actions on vertebrate sexual development via additional mechanisms that enhance the effects of aromatase inhibition alone. On the other hand, direct comparison is hindered by the fact that prochloraz along with other pesticides have additional endocrine modulating properties (i.e., androgen and estrogen receptor interactions). A thorough review of these data is required before firm conclusions can be made on the primary adverse outcome pathway of TBT in vertebrates.

There were various effects (including in vitro changes in genomic markers and enzyme activities) in other fish species within at least one order of magnitude of the results obtained by McAllister and Kime (2003) but these endpoints and LOEC values for zebrafish stand out as the most ecotoxicologically relevant. This suggests that the endpoints used for other fish studies (often growth) were not the most relevant. However, the dramatic effect observed in zebrafish may be restricted to fish species that share the same pattern for sexual differentiation rather than all fish. Zebrafish gonads initially develop as ovaries, however in male fish, the ovarian tissue degenerates and the testis develop (Maack and Segner 2003). This period of juvenile hermaphroditism (Takahashi 1977) may explain the increased sensitivity of the sex ratio endpoint after exposure to AR agonists and aromatase inhibitors during critical developmental windows.

7.2 Tissue Residue-Based Analysis

A given ambient toxicity metric (e.g., EC_{50} or LOEC) that is based on aqueous or sediment concentrations can result in a range spanning orders of magnitude for different species. The equivalent tissue residue toxicity metric (e.g., ER_{50} or LOER) often exhibits lower variability (Meador 1997). This has been observed for a large variety of taxa and chemicals, which has been discussed in many publications (Meador 2006; Meador et al. 2008; McElroy et al. 2011) and is known as the tissue-residue approach for toxicity assessment. TBT provided one of the first examples of the utility for this approach based on a large database of tissue residue toxicity data for mortality and reduced growth. The analysis of TBT toxicity has expanded from evaluating these high dose endpoints to endocrine-related responses in invertebrates and fish responding to very low environmental concentrations. While some of the datasets are limited, they do indicate a relatively consistent response among species for a given endpoint and whole-body tissue concentration (Meador 2011). Widely variable toxicokinetics among species is the main factor responsible for the high interspecies variability for a given toxicity metric based on external dose. Consequently, when internal dose is used to determine a toxicity value, toxicokinetic differences are not a confounding factor, which results in greatly reduced variability among species response values (Meador et al. 2008; McCarty et al. 2011).

An analysis of the imposex endpoint as a function of whole-body tissue concentrations is shown in Fig. 6. The program SSDMaster (Rodney and Moore 2008) was

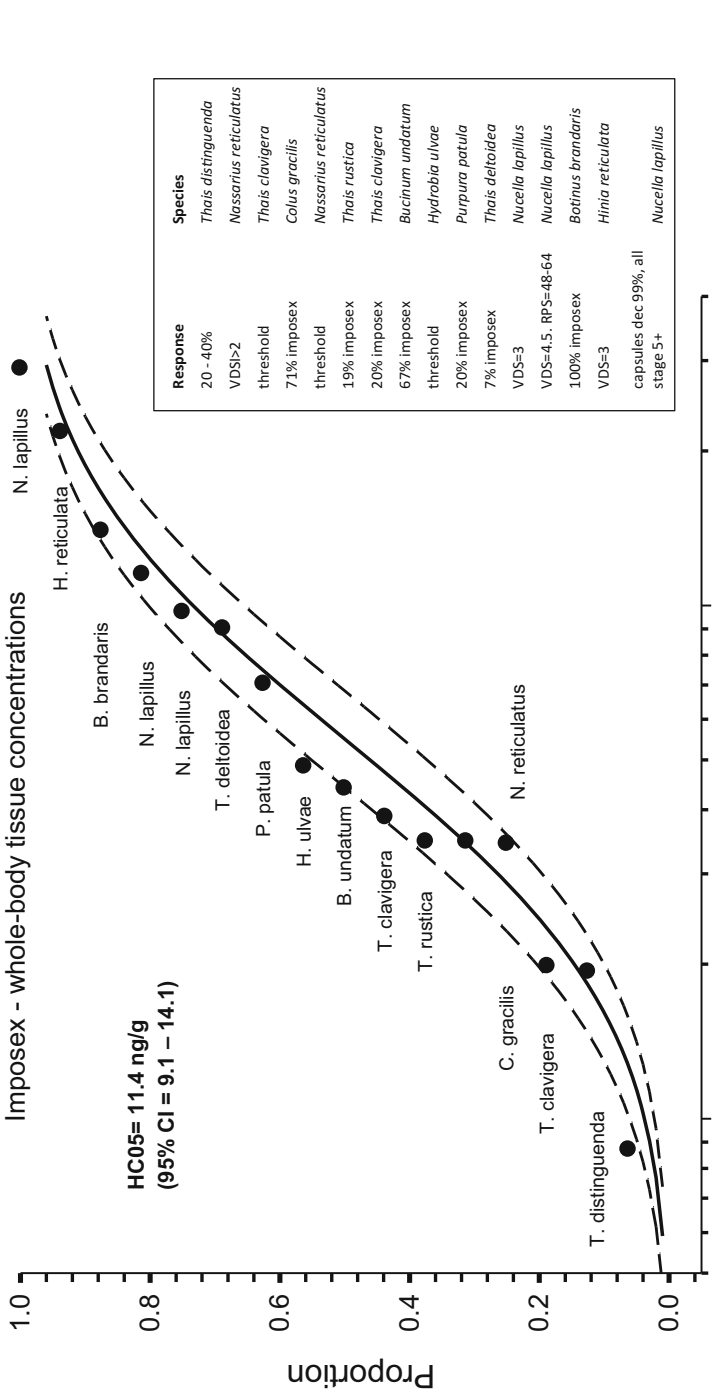


Fig. 6 Cumulative distribution function (CDF) constructed with tissue TBT concentrations (as ng TBT/g wet weight) that induce imposex in Caenogastropod molluscs

used to generate the data for this plot. The cumulative distribution function (CDF) shows that the sensitivity of one given species may vary according to the imposex stage and TBT tissue burden. These data indicate a relatively narrow range of concentrations for the imposex response spanning from threshold to 100% induced. Based on these data, the hazard concentration resulting in low level effects was determined to be 11.4 ng TBT/g wet weight (95% CI = 9.1–14.1). This concentration characterizes a low level response; hence, a safety factor may be needed to determine the potential "no effect" level. The tissue concentrations in this CDF have utility in assessing population fitness for these gastropods in the field.

8 Sources of Uncertainty, Data Gaps, and Confounding Issues

8.1 Transgenerational Effects

TBT is highly bioaccumulative and maternal transfer to eggs has been demonstrated (e.g., Inoue et al. 2006; Ohji et al. 2006). Effects can occur over multiple generations in some invertebrate species, e.g. chironomids (Lilley et al. 2012) and copepods (Huang et al. 2006), as well as in fish, birds, and mammals. However, it is not clear whether or not this is exclusively linked to endocrine-mediated mechanisms.

8.2 Sensitive Species

The general perception is that molluscs are the most sensitive taxonomic group to TBT exposure due to the observation of imposex in wild species at low environmental concentrations. However, the present analysis determined that a number of fish species and other invertebrate species (e.g., copepods) have similar or greater levels of sensitivity when considered in terms of population-relevant responses.

8.3 Potency

With respect to endocrine-mediated effects, TBT is highly potent as it can act in aquatic organisms at levels of parts per trillion (ng/L) and lower (Figs. 4 and 5), and low ng/g tissue concentrations (Fig. 6), whereas lethal toxicity occurs at much higher concentrations. Thus, the HC5 value derived from an SSD based on LOECs for mortality (Fig. 7) is ten times higher than the HC5 estimated from LOEC for sublethal effects (Fig. 4). In the copepod *Acartia tonsa*, the NOEC values for

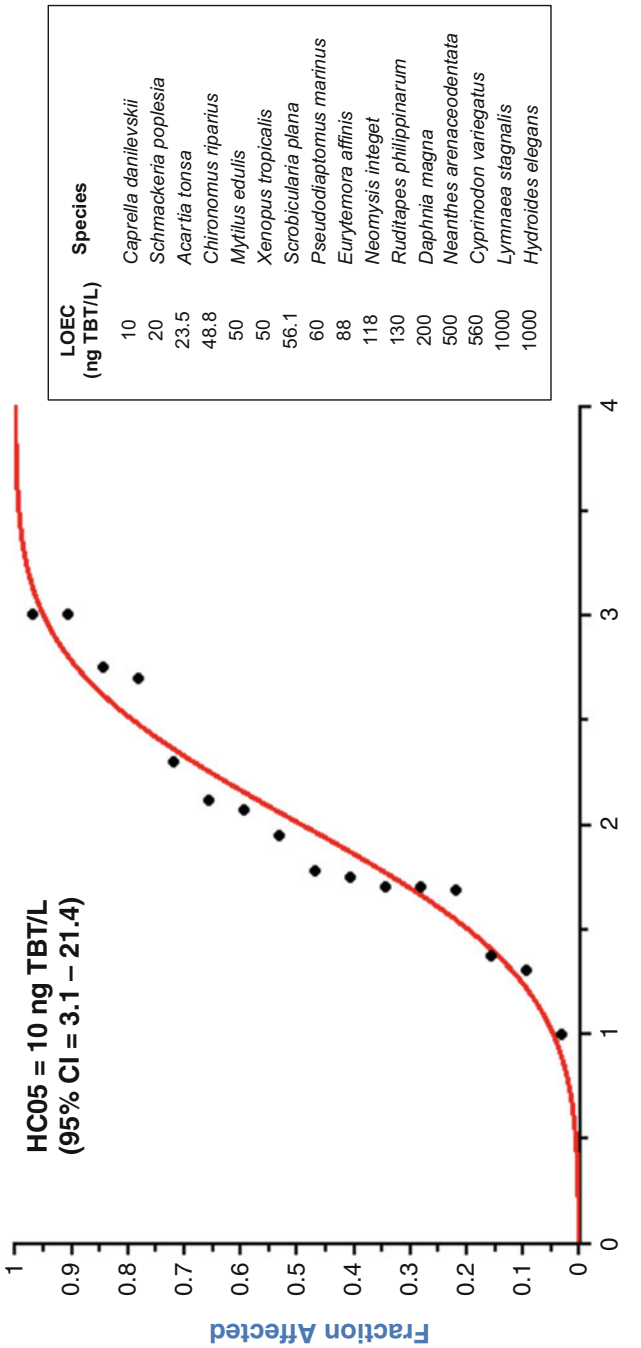


Fig. 7 Species sensitivity distribution (SSD) constructed with TBT LOEC values for mortality in 16 most sensitive aquatic species. Data that were used to construct this SSD are given in Table 4 of the Annexes). The goodness-of-fit was acceptable (significance level: 0.01–0.1), as assessed using the Anderson-Darling, Kolmogorov-Smirnov, and Cramer von Mises tests for normality

reproductive effects and mortality are 0.7 and 11 ng/L, respectively (Kusk and Petersen 1997).

Potency of TBT is also shown at the molecular level where molecular initiating events are elicited at concentrations that are several hundred times lower than those causing basal toxicity (Fig. 3).

8.4 Non-monotonic Dose-Response or Lack of a Threshold Dose

Inverted U-shape responses to TBT have been observed for some endpoints, depending on the concentration, mainly involving gene expression studies (e.g., Mortensen and Arukwe 2007; Kortner et al. 2010; Morales et al. 2013; Pascoal et al. 2013). From in vivo studies, there is also some evidence that TBT has been shown to display an inverted U-shape response for several endpoints. A good example is the impact of TBT on body weight in fish and mammals (Cooke et al. 2008; Meador et al. 2011; Si et al. 2011). Meador et al. (2011), for example, found that TBT exposure in fish enhanced growth and lipogenesis at low doses and inhibited growth and reduced lipid content at high doses; this was attributed to two modes of action operating at different doses. Most likely, the cause of a non-linear response for TBT is different dose-dependent mechanisms of action. TBT is known to be an uncoupler of oxidative phosphorylation and directly affects ATPase (ATP-synthase). This enzyme that plays an important role for providing cellular energy is located within the mitochondrial membrane and consists of two regions: the Fo section, embedded within the membrane and the F_1 section, outside the membrane but inside the matrix of the mitochondria. TBT interacts with the Fo section of ATPase. When exposures occur at higher doses, this is the most likely MeOA that causes reduced growth and death. Baseline toxicity (narcosis) is not possible because all species die at about 10^4 ng/g whole-body concentration, far below baseline doses. Hence, the low dose effects are definitely due to RXR-PPAR binding but high dose responses are due to mitochondrial dysfunction. Of course, the U-shaped response will depend on the endpoint; it is highly plausible for metabolic effects. The question is whether this non-linear behavior will occur for other responses. Such non-linear concentration-response should be considered with caution regarding the magnitude and consistency of the changes. It is important to determine whether or not these responses are significant, and if they consistently occur in different species. One should also consider whether U-shaped dose-responses are a result of endocrine-mediated perturbations, multiple mechanisms of action, or adaptive process (see Parrott et al. 2017, and the references therein). They also may relate to exposure artifacts (e.g., exceeding chemical solubility, erroneous doses, or failure of chemical delivery systems).

9 Areas for Future Research

Although TBT has now been recognized as an EDC at a global scale, if its ED properties were originally assessed at low concentrations using a limited group of USEPA Tier-1 tests that did not include the AMA, further testing may not have been triggered. The current Tier-1 tests cover primarily three hormonal pathways (EAT) and as such do not target the primary endocrine mode of action of TBT, via RXR/PPAR activation. Based on the literature reviewed here, TBT would elicit positive results in the steroidogenesis and aromatase assays only at levels much higher than those needed to elicit an endocrine effect via the RXR/PPAR interactions. The lack of testing for other hormonally and metabolically important nuclear receptors such as PPAR and RXR under both the US and OECD testing batteries has been recognized as a deficiency and prioritized for assay development by OECD (OECD 2012a). The results of the present review underscore this need. However, we recognize that assays for RXR/PPAR activity may be more difficult to develop and validate than those for other nuclear receptors such as ER and AR, due to the complex nature of their activation, the formation of homo- and heterodimers with other nuclear receptors, and the permissive/non-permissive nature of downstream effects. In other words, a different platform than the current transactivation assays (e.g., one that allows assessing cross-talk between these receptors) may be needed for a full understanding of perturbations stemming from chemical interactions with these receptors.

In addition to new low-tier testing that goes beyond EAT, suitable higher tier tests should also be developed in order to assess the plethora of biological effects that involve targets other than EAT. To this end, the new OECD Test Guidelines (TG242 and TG243) on molluscan reproductive toxicity are an important development, although such tests do not discriminate between ED and non-ED mediated mechanisms. However, standardized partial or full life-cycle tests should also be developed for additional invertebrate phyla such as annelids and echinoderms as they are not only numerous, but also ecologically important taxonomic groups with a largely unknown endocrine system.

When designing a testing strategy for chemicals that are suspected to act via the endocrine system, ecotoxicologists should incorporate the lessons learned from TBT, as they are both numerous and important. The first relates to apparent species sensitivity. Previously, molluscs were assumed to be the most sensitive group to TBT, primarily due to overt population effects. However, our analyses demonstrated that certain fish species are equally if not more sensitive to its effects. Likewise, the body burden of a chemical should be taken into account especially when the apparent sensitive species are filter feeders. In many cases, these species exhibit elevated bioaccumulation because of higher rates of uptake and lower rates of metabolism compared to other species. Tissue residue toxicity metrics also vastly improve the characterization of toxic responses because the inherent variability in toxicokinetics found among species is incorporated and accounted for.

Another important aspect relates to species extrapolation, particularly when fundamental aspects of endocrine control are largely unknown. TBT is not the only chemical with observed effects in invertebrate species that were subject to erroneous interpretations based on vertebrate endocrinology. The same assumption was made for the role of estrogens on bivalve vitellogenesis (Gagné et al. 2001), which resulted in numerous research programs globally attempting to use bivalve molluscs as model species for studying effects of vertebrate steroids (Scott 2012, 2013). The presence of an estrogen-like receptor in their genome cemented this assumption without any functional characterization of this receptor (Kishida et al. 2005). Only recently this assumption was proven incorrect (Morthorst et al. 2014), highlighting yet again the importance of fundamental knowledge before a sound testing strategy is in place.

Finally, another important lesson learned from TBT is that care should be taken when dealing with chemicals that display multiple mechanisms of action; the actual number of these is unknown but they do exist and can lead to incorrect interpretations of experimental and field data. Retrospective analysis of the TBT data clearly indicates a dual mode of toxicity (low versus high doses) that is a function of different MeOA. This highlights the need for comprehensive testing at different levels and using different species prior to interpretation of data.

10 Conclusions and Recommendations

Environmental TBT concentrations measured post-2008 show that exposure may still occur in the range 0.1–8 ng/L (mean = 0.3 ng/L) in representative European surface waters (UK Environment Agency, pers. com. 2016) and in other locations (e.g., Kim et al. 2014; Ho et al. 2016), suggesting that there is still an environmental risk from legacy contamination. Risks may be higher in regions of the world with less effective enforcement. TBT was introduced on the market in the early 1960s, at a time when regulatory assessment of chemicals was at its infancy. Using deliberate "retrospective thinking," and considering the information gathered in this case study, one important question arises: would TBT be identified as an ED using current screening and testing methods?

Typical endocrine responses are elicited by compounds that mimic estrogen, androgen, and thyroid pathway hormones and act via various nuclear receptors. Tributyltin is not considered a classic endocrine disruptor, because it impacts reproductive and metabolic pathways primarily through interaction with the retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPARγ) nuclear receptors.

Using assays recommended in the OECD CFEDTA, TBT was shown to alter the sex-ratio and to induce sperm abnormality in the Fish Sexual Development Test (FSDT) with zebrafish (McAllister and Kime 2003), and to delay frog development in the AMA (Shi et al. 2014), at low concentrations (0.1 and 10 ng/L, respectively). It also appeared positive in a FETAX-like assay (Guo et al. 2010). Based upon these

findings, TBT would be identified as acting on endocrine pathways, although the specific MeOA (RXR and/or PPAR) would remain unknown. However, refinements to ToxCast™ now allow the identification of endocrine molecular initiating events through RXR and/or PPAR pathways. TBT activity for in vitro RXR and PPAR assays typically occurs at levels far less than those for baseline toxicity.

Interestingly, a more thorough evaluation of the available data clearly shows that TBT is highly toxic to a variety of aquatic taxa. Through a comparative analysis of the potency of TBT in various aquatic species, our review highlights the observation that fish are as sensitive, or more so, compared to molluscs when based on water exposure. This is an important conclusion because molluscs were long recognized as uniquely sensitive to this compound. TBT's precise MeOA is still incompletely understood but may include link/cross-talk between PPARs (i.e., carbohydrate, lipid, protein metabolism), RXRs (i.e., development), thyroid (growth) and even sex determination and differentiation pathways; the latter pathways may be stronger affected by TBT exposure in species where environmental factors play a significant role in determining sex ratios (e.g., zebrafish).

Current screening and assessment methodologies are able to identify TBT as a potent endocrine disruptor with a high environmental risk. If those approaches were available when TBT was introduced to the market, it is likely that its use would have been regulated sooner, thus avoiding the detrimental effects on marine gastropod populations and communities as documented over several decades.

This retrospective evaluation of TBT, a very potent endocrine disruptor in vertebrates and invertebrates, should serve as an example demonstrating how shortfalls within the framework of chemical toxicity evaluation can result in under-protective regulatory assessment. Nowadays, the assays included in the OECD Conceptual Framework, including those recently developed on gastropod molluscs would likely recognize TBT as a chemical of concern with respect to endocrine disruption, although its mechanism of action and potency across taxonomic groups would remain largely unknown. Reflective analysis of well-studied, but potentially misunderstood contaminants, such as TBT, provides important lessons that should serve as a guiding principle for future studies and refinements of assessment protocols.

11 Summary

Tributyltin (TBT) has been recognized as an endocrine disrupting chemical (EDC) for several decades. However, only in the last decade, was its primary endocrine mechanism of action (MeOA) elucidated—interactions with the nuclear retinoid-X receptor (RXR), peroxisome proliferator-activated receptor γ (PPARγ), and their heterodimers. This molecular initiating event (MIE) alters a range of reproductive, developmental, and metabolic pathways at the organism level. One of the most important lessons learned from years of research on TBT concerns apparent species sensitivity. Several aspects such as the rates of uptake and elimination, chemical

potency, and metabolic capacity are all important for identifying the most sensitive species for a given chemical, including EDCs. As recognized for many years, TBT-induced responses are known to occur at very low concentrations for molluscs, a fact that has more recently also been observed in fish species. This review explores the MeOA and effects of TBT in different species (aquatic molluscs and other invertebrates, fish, amphibians, birds and mammals) according to the OECD Conceptual Framework for Endocrine Disruptor Testing and Assessment (CFEDTA). The information gathered on biological effects that are relevant for populations of aquatic animals was used to construct Species Sensitivity Distributions (SSDs) based on No Observed Effect Concentrations (NOECs) and Lowest Observed Effect Concentrations (LOECs). Fish appear at the lower end of these distributions, showing that they are as sensitive as molluscs, and for some species, even more sensitive. Concentrations in the range of 1 ng/L for water exposure (10 ng/g for whole-body burden) have been shown to elicit endocrine-type responses, whereas mortality occurs at water concentrations ten times higher. Current screening and assessment methodologies as compiled in the OECD CFEDTA are able to identify TBT as a potent endocrine disruptor with a high environmental risk for the original use pattern. If those approaches had been available when TBT was introduced to the market, it is likely that its use would have been regulated sooner, thus avoiding the detrimental effects on marine gastropod populations and communities as documented over several decades.

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Disclaimer

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Conflict of Interest

The authors declare that they have no conflict of interest.

Annexes

Table 1 Updated OECD conceptual framework for testing and assessment of endocrine disrupters (OECD 2012a, b)

Level	Mammalian and non-mammalian toxicology
1	• Physical & chemical properties, e.g., MW reactivity, volatility, biodegradability
Existing data and non-test information	• All available (eco)toxicological data from standardized or non-standardized tests
	• Read across, chemical categories, QSARs and other in silico predictions, and ADME model predictions
2	• Estrogen or androgen receptor binding affinity

(continued)

Level	Mammalian and non-mammalian toxicology	
In vitro assays providing data about selected endocrine mechanism(s)/pathways(s)	• Estrogen receptor transactivation (TG 455–TG 457)	
	• Androgen or thyroid transactivation (if/when TGs are available)	
	• Steroidogenesis in vitro (TG 456)	
	• MCF-7 cell proliferation assays (ER ant/agonist)	
	• Other assays as appropriate	
	Mammalian toxicology	Non-mammalian toxicology
3 In vivo assays providing data about selected endocrine mechanism(s)/pathway(s)[a]	• Uterotrophic assay (TG 440)	• Xenopus embryo thyroid signaling assay (when/if TG is available)
		• Amphibian metamorphosis assay (TG 231)
	• Hershberger assay (TG 441)	• Fish reproductive screening assay (TG 229)
		• Fish screening assay (TG 230)
		• Androgenized female stickleback screen (GD 140)
4	• Repeated dose 28-day study (TG 407)	• Fish sexual development test (TG 234)
In vivo assays providing data on adverse effects on endocrine relevant endpoints[b]	• Repeated dose 90-day study (TG 408)	• Fish reproduction partial life-cycle test (when/if TG is available)
	• One-generation reproduction toxicity study (TG 415)	• Larval amphibian growth & development assay (when TG is available)
	• Male pubertal assay (see GD 150, C4.3)[c]	
	• Female pubertal assay (see GD 150, C4.4)[c]	• Avian reproduction assay (TG 206)
		• Mollusc reproduction test (TG 242–TG 243, adopted 2016)[d]
	• Intact adult male endocrine screening assay (see GD 150, Annex 2.5)	• Chironomid toxicity test (TG 218–219)[d]
		• Daphnia reproduction test (with male induction) (TG 211)[d]
	• Prenatal developmental toxicity study (TG 414)	• Earthworm reproduction test (TG 222)[d]
	• Chronic toxicity and carcinogenicity studies (TG 451-3)	• Enchytraeid reproduction test (TG 220)[d]
	• Reproductive screening test (TG 421 if enhanced)	• Sediment water Lumbriculus toxicity test using spiked sediment (TG 225)[d]
	• Combined 28-day/reproductive screening assay (TG 422 if enhanced)	• Predatory mite reproduction test in soil (TG 226)[d]
		• Collembolan reproduction test in soil (TG 232)[d]
	• Developmental neurotoxicity (TG 426)	

(continued)

Level	Mammalian and non-mammalian toxicology	
5	• Extended one-generation reproductive toxicity study (TG 443)[e]	• Medaka extended one-generation reproduction test (MEOGRT) (TG 240)
In vivo assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism[b]	• Two-generation reproduction toxicity study (TG 416 most recent update)	• FLCTT (fish life-cycle toxicity test) (when TG is available)
		• Avian two-generation reproductive toxicity assay (when TG is available)
		• Mysid life-cycle toxicity test (when TG is available)[d]
		• Copepod reproduction and development test (when TG is available)[d]
		• Sediment water chironomid life-cycle toxicity test (OECD TG 233)[d]
		• Mollusc full life-cycle assays (when TG is available)[d]

[a]Some assays may also provide some evidence of adverse effects
[b]Effects can be sensitive to more than one mechanism and may be due to non-ED mechanisms
[c]Depending on the guideline/protocol used, the fact that a substance may interact with a hormone system in these assays does not necessarily mean that when the substance is used it will cause adverse effects in humans or ecological systems
[d]At present, the available invertebrate assays solely involve apical endpoints which are able to respond to some endocrine disrupters and some non-EDs. Those in Level 4 are partial life-cycle tests, while those in Level 5 are full- or multiple life-cycle tests
[e]The Extended one-generation reproductive toxicity study (TG 443) is preferable for detecting endocrine disruption because it provides an evaluation of a number of endocrine endpoints in F1 juvenile and adult, which are not included in the two-generation study (TG 416 adopted 2001)

Table 2 LOEC (lowest observed effect concentration) of TBT for population-relevant endpoints in aquatic organisms

Species	Test conditions	Biological endpoint	LOEC (ng TBT/L)	Reported as[a]	Reference
Amphibians					
Xenopus laevis	AMA (stage 51 tadpoles)/CAMA (stage 46 tadpoles)	Embryo body length and development	10.00	nom	Shi et al. (2014)
Xenopus tropicalis	FETAX-like test	Tadpole development	50.00	nom	Guo et al. (2010)
Fish					
Carassius auratus	Adults, 54 days (semi-static exposure)	Body weight, swimming activity	2.15	mm	Zhang et al. (2016)
Danio rerio	Full life-cycle test	Sperm abnormality 3–5 months post-exposure	0.10	nom	McAllister and Kime (2003)
Danio rerio	Larvae, 0–70 days post-hatch	Sex ratio	0.10	nom	McAllister and Kime (2003)
Fundulus heteroclitus	Full life-cycle test	Sex ratio F_1	420.00	mm	Mochida et al. (2010)
Fundulus heteroclitus	Full life-cycle test	Time to hatch F_1	750.00	mm	Mochida et al. (2010)
Oryzias latipes	Embryos	Hatching success	10,440.00	mm?	Bentivegna and Piatkowski (1998)
Pimephales promelas	Early life-stage test, 32 days	Fry growth and weight	450.00	mm?	Brooke et al. (2003)
Poecilia reticulata	Male adults, 28 days	Reproductive behavior	5.00	nom	Tian et al. (2015)
Sebastiscus marmoratus	Embryos (gastrula stage), 144 h	Hatchability	10.00	nom	Zhang et al. (2011)
Crustaceans					
Acanthomysis sculpta	Life-cycle test	Reproduction	190.00	mm	Davidson et al. (2003)
Caprella danilevskii	Full life-cycle test	Sex ratio	100.00	nom	Ohji et al. (2002)
Daphnia magna	F_0 third-instar juveniles (3 days) and F_1 egg provisioning stage	Reproduction	88.00	mm	Jordão et al. (2015)
Daphnia magna	Adults, 21 days	Offspring/female	2500.00	nom	Oberdorster et al. (1998)

(continued)

Table 2 (continued)

Species	Test conditions	Biological endpoint	LOEC (ng TBT/L)	Reported as[a]	Reference
Pseudodiaptomus marinus	Full life-cycle test	F_0 fecundity (nauplii/female)	60.00	nom	Huang et al. (2006)
Pseudodiaptomus marinus	Full life-cycle test	Sex ratio F_1	20.00	nom	Huang et al. (2006)
Pseudodiaptomus marinus	Full life-cycle test	Ovigerous F_0 females (%)	60.00	nom	Huang et al. (2006)
Schmackeria poplesia	Full life-cycle test	Ovigerous females (%)	10.00	nom	Huang et al. (2010)
Schmackeria poplesia	Full life-cycle test	Larval development	60.00	nom	Huang et al. (2010)
Tigriopus japonicus	Ovigerous females, 14 days	Nauplii production	50.00	nom	Ara et al. (2010)
Molluscs					
Biomphalaria glabrata	From hatchlings to adults, prolonged exposure	Egg laying	1.00	nom	Ritchie et al. (2005)
Crassostrea gigas	Oyster spats, 28 days	Growth (weight gain)	5.00	nom?	Nell and Chvojka (1992)
Lymnaea stagnalis	Adults, 21 days	Polyembryony	46.36	mm	Giusti et al. (2013b)
Lymnaea stagnalis	Adults, 56 days	Fecundity	372.82	gmm	Charles et al. (2016)
Lymnaea stagnalis	Adults, 21 days	Growth (shell size)	481.47	mm	Giusti et al. (2013b)
Lymnaea stagnalis	Eggs, 21 days	Mean hatching time	100.00	nom	Bandow and Weltje (2012)
Lymnaea stagnalis	Adults, 170 days	Fecundity	1000.00	nom	Leung et al. (2007)
Nucella lapillus	Egg capsule to adults, 1 year	Reproduction	27.80	mm	Harding et al. (2003)
Marisa cornuarietis	Adults, 8 weeks	Embryo production	39.50	nom	Schulte-Oehlmann (1997)
Mercenaria mercenaria	Veliger larvae, 14 days static renewal	Growth (shell size)	5.00	nom	Laughlin et al. (1988)
Mytilus edulis	Larvae, 33 days	Growth (shell size)	50.00	nom	Lapota et al. (1993)

Potamopyrgus antipodarum	Adults, 28 days	Fecundity	184.71	mm	Ruppert et al. (2016)
Pinctada fucata martensii	One-week static renewal exposure of adult females	Embryo development	191.00	mm	Inoue et al. (2004)
Pinctada fucata martensii	24-h static exposure of fertilized eggs	Embryo development	192.00	mm	Inoue et al. (2004)
Ruditapes philippinarum	24-h static exposure of fertilized eggs	Embryo development	62.00	mm	Inoue et al. (2006)
Saccostrea commercialis	Oyster spats, 28 days	Growth (weight gain)	5.00	nom?	Nell and Chvojka (1992)
Scrobicularia plana	30-day static renewal exposure of 10-day old pediveliger larvae	Larval shell growth	30.00	mm	Ruiz et al. (1995)
Echinoderms					
Paracentrotus lividus	fertilized eggs, 48 h	Embryo development and larval length	200.00	nom	Bellas et al. (2005))
Ascidians					
Ciona intestinalis	Embryos (2-cell stage), 20 h	Embryo development	4000.00	nom	Bellas et al. (2005)
Ciona intestinalis	Exposure of oocytes and spermatozoa	Fertilization rate	290,000.00	nom	Gallo and Tosti (2013)

[a]*nom* nominal, *mm* arithmetic mean measured, *gmm* geometric mean measured
For each species, the lowest LOEC value was used to construct the SSD (Fig. 4)

Table 3 NOEC (no observed effect concentration) of TBT for population-relevant endpoints in aquatic organisms

Species	Test duration	Biological endpoint	NOEC (ng TBT/L)	Reported as[a]	Reference
Fish					
Cyprinodon variegatus	Juveniles, 24 days	Growth	340.00	nom?	De Bruijn et al. (2005)
Danio rerio	Full life-cycle test	Sperm abnormality 3–5 months post-exposure	0.01	nom	McAllister and Kime (2003)
Danio rerio	Larvae, 0–70 days post-hatch	Sex ratio	0.01	nom	McAllister and Kime (2003)
Fundulus heteroclitus	Full life-cycle test	Sex ratio F_1	70.00	mm	Mochida et al. (2010)
Fundulus heteroclitus	Full life-cycle test	Hatchability F_0 (%)	750.00	mm	Mochida et al. (2010)
Fundulus heteroclitus	Full life-cycle test	Time to hatch F_1	520.00	mm	Mochida et al. (2010)
Gasterosteus aculeatus	225 days	Reproduction	100.00	mm?	De Bruijn et al. (2005)
Oncorhynchus mykiss	Juveniles, 16 weeks	Growth	60.00	nom?	De Bruijn et al. (2005)
Pimephales promelas	Early life-stage test, 32 days	Fry growth and weight	150.00	mm?	Brooke et al. (2003)
Poecilia reticulata	Juveniles, 91 days	Growth	320.00	nom?	De Bruijn et al. (2005)
Sebastiscus marmoratus	Embryos (gastrula stage), 144 h	Hatchability	1.00	nom	Zhang et al. (2011)
Crustaceans					
Acanthomysis sculpta	Life-cycle test	Reproduction	90.00	mm	Davidson et al. (2003)
Acartia tonsa	Eggs, 8 days	Larval development EC_{10}	0.70	nom	Kusk and Petersen (1997)
Caprella danilevskii	Full life-cycle test	Sex ratio	10.00	nom	Ohji et al. (2002)
Daphnia magna	Adults, 21 days	Reproduction	160.00	mm?	Kühn et al. (2005)
Daphnia magna	Adults, 21 days	Offspring/female	1250.00	nom	Oberdorster et al. (1998)

Eurytemora affinis	Adults, 13 days	Reproduction	10.00	mm?	De Bruijn et al. (2005)
Eurytemora affinis	Egg-carrying females, 13 days	Brood size	224.00	mm	Hall et al. (2003)
Pseudodiaptomus marinus	Full life-cycle test	Sex ratio F_1	6.00	nom	Huang et al. (2006)
Pseudodiaptomus marinus	Full life-cycle test	Ovigerous F_0 females (%)	20.00	nom	Huang et al. (2006)
Pseudodiaptomus marinus	Full life-cycle test	F_0 fecundity (nauplii/female)	20.00	nom	Huang et al. (2006)
Schmackeria poplesia	Full life-cycle test	Ovigerous females (%)	5.00	nom	Huang et al. (2010)
Schmackeria poplesia	Full life-cycle test	Larval development	40.00	nom	Huang et al. (2010)
Tigriopus japonicus	Ovigerous females, 14 days	Nauplii production	25.00	nom	Ara et al. (2010)
Molluscs					
Isognomon californicum	Gametes 48 h	Fertilization rate	1000.00	nom	Ringwood (1992)
Isognomon californicum	Embryos, 48 h	Embryo development	100.00	nom	Ringwood (1992)
Isognomon californicum	Veliger larvae, 4 days	Larval growth	20.00	nom	Ringwood (1992)
Lymnaea stagnalis	Adults, 170 days	Fecundity	10.00	nom	Leung et al. (2007)
Lymnaea stagnalis	Adults, 170 days	Population growth rate	2745.00	nom	Leung et al. (2007)
Lymnaea stagnalis	Adults, 56 days	Fecundity	231.00	gmm	Charles et al. (2016)
Lymnaea stagnalis	Adults, 21 days	Growth (shell size)	229.74	mm	Giusti et al. (2013a)
Lymnaea stagnalis	Eggs, 21 days	Mean hatching time	30.00	nom	Bandow and Weltje (2012)
Nucella lapillus	Egg capsule to adults, 1 year	Reproduction	7.40	mm	Harding et al. (2003)
Mytilus edulis	Larvae, 33 days	Growth (shell size)	6.00	nom	Lapota et al. (1993)
Pinctada fucata martensii	One-week static renewal exposure of adult females	Embryo development	92.00	mm	Inoue et al. (2004)

(continued)

Table 3 (continued)

Species	Test duration	Biological endpoint	NOEC (ng TBT/L)	Reported as[a]	Reference
Pinctada fucata martensii	24-h static exposure of fertilized eggs	Embryo development	91.00	mm	Inoue et al. (2004)
Potamopyrgus antipodarum	Adults, 28 days	Fecundity	95.65	mm	Ruppert et al. (2016)
Echinoderms					
Echinometra mathaei	Gametes, 60–90 min	Fertilization rate	1000.00	nom	Ringwood (1992)
Ophioderma brevispina	28 days	Regeneration	10.00	mm?	Walsh et al. (2005)
Paracentrotus lividus	Fertilized eggs, 48 h	Embryo development and larval length	100.00	nom	Bellas et al. (2005)
Ascidians					
Ciona intestinalis	Embryos (2-cell stage), 20 h	Embryo development	2000.00	nom	Bellas et al. (2005)
Annelids					
Neanthes arenaceodentata	Adults, 70 days	Growth	50.00	nom?	Moore et al. (2003)

[a]*nom* nominal, *mm* arithmetic mean measured, *gmm* geometric mean measured
For each species, the lowest NOEC value was used to construct the SSD (Fig. 5)

Table 4 LOEC (lowest observed effect concentration) of TBT for mortality in aquatic organisms

Species	Test duration	Biological endpoint	LOEC (ng TBT/L)	Reported as[a]	Reference
Amphibians					
Xenopus tropicalis	FETAX-like test, 48 h	Tadpole development	50.00	nom	Guo et al. (2010)
Fish					
Cyprinodon variegatus	n.r.	Parental survival	560.00	mm?	United States Environmental Protection Agency - US EPA (2008)
Oryzias latipes	Embryonic stages, 96 h	Embryo survival	41,500.00	mm?	Bentivegna and Piatkowski (1998)
Crustaceans					
Acartia tonsa	Nauplii larvae, 6 days	Larval survival	23.50	mm	Bushong et al. (1990)
Caprella danilevskii	Full life-cycle test	Embryo survival	10.00	nom	Ohji et al. (2003b)
Daphnia magna	21-day chronic	Survival and reproduction	200.00	mm?	Brooke et al. (2003))
Daphnia magna	21-day chronic	Survival and reproduction	340.00	mm?	ABC Laboratories Inc. (2003)
Daphnia magna	21-day chronic run over two generations	Adult survival	2225.00	nom	Oberdorster et al. (1998)
Eurytemora affinis	n.r.	Neonate survival	88.00	mm?	Hall et al. (2003)
Palaemon serratus	Zoe I stage larvae, 48 h	Larval survival	62,500.00	nom	Bellas et al. (2005)
Pseudodiaptomus marinus	Nauplii to copepodites, 13 days	Larvae to adult survival F_0	60.00	nom	Huang et al. (2006)
Schmackeria poplesia	Full life-cycle test	Adult survival	20.00	nom	Huang et al. (2010)
Tisbe biminiensis	Adult (7–10 day-old), 48 h	Adult survival	34,000.00	nom	Varella Motta da Costa et al. (2014)
Molluscs					
Lymnaea stagnalis	Adults, 170 days	Adult survival	1000.00	nom	Leung et al. (2007)
Lymnaea stagnalis	Adults, 170 days	Juvenile survival	1000.00	nom	Leung et al. (2007)

(continued)

Table 4 (continued)

Species	Test duration	Biological endpoint	LOEC (ng TBT/L)	Reported as[a]	Reference
Mytilus edulis	33 days	Larval survival	50.00	nom	Lapota et al. (1993)
Ruditapes philippinarum	Static renewal exposure of veliger larvae (D-larvae stage) for 13 days	Survival and development of veliger larvae	130.00	mm	Inoue et al. (2007)
Scrobicularia plana	30-day static renewal exposure of 10-day-old pediveliger larvae	Larval survival	56.10	mm	Ruiz et al. (1995)
Insects					
Chironomus riparius	Fourth instar larvae, 48 h	Larval survival	48.80	nom	Hahn and Schulz (2002)
Annelids					
Hydroides elegans	Early development (egg to juvenile)	Adult (post-spawning female) survival	10,000.00	nom	Lau et al. (2007)
Hydroides elegans	Early development (egg to juvenile)	Juvenile survival	1000.00	nom	Lau et al. (2007)
Neanthes arenaceodentata	Adults, 70 days	Adult survival	500.00	nom?	Moore et al. (2003)

[a]*nom* nominal, *mm* arithmetic mean measured, *gmm* geometric mean measured

For each species, the lowest LOEC value was used to construct the SSD (Fig. 7). Species for which LOEC values were higher than 1000 ng/L were not considered sensitive and were not included in the SSD

References

ABC Laboratories Inc. (2003) 1990 in United States Environmental Protection Agency. Ambient aquatic life water quality criteria for tributyltin (TBT) – final. Office of Water, Office of Science and Technology Health and Ecological Criteria Division, Washington, DC. 129 pages

Abidli S, Santos MM, Lahbib Y, Castro LFC, Reis-Henriques MA, El Menif NT (2012) Tributyltin (TBT) effects on *Hexaplex trunculus* and *Bolinus brandaris* (Gastropoda: Muricidae): imposex induction and sex hormone levels insights. Ecol Indic 13:13–21

Abidli S, Castro LFC, Lahbib Y, Reis-Henriques MA, El Meni NT, Santos MM (2013) Imposex development in *Hexaplex trunculus* (Gastropoda: Caenogastropoda) involves changes in the transcription levels of the retinoid X receptor (RXR). Chemosphere 93:1161–1167

Alzieu C (2000) Environmental impact of TBT: the French experience. Sci Total Environ 258:99–102

Alzieu C, Sanjuan J, Deltriel J-P, Borel M (1986) Tin contamination in Arcachon bay: effects on oyster shell anomalies. Mar Pollut Bull 17:494–498

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA, Tietge JE, Villeneuve DL (2010) Adverse Outcome Pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29:730–741

Antizar-Ladislao B (2008) Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. Environ Int 34:292–308

Arnold CG, Weidenhaupt A, David MM, Müller SR, Haderlein SB, Schwarzenbach RP (1997) Aqueous speciation and 1-octanol-water partitioning of tributyl- and triphenyltin: effect of pH and ion composition. Environ Sci Technol 31:2596–2602

Avaca MS, Martin P, van der Molen S, Narvarte M (2015) Comparative study of the female gametogenic cycle in three populations of *Buccinanops globulosus* (Caenogastropoda: Nassariidae) from Patagonia. Helgol Mar Res 69:87–99

Bailey SK, Davies IM, Harding MJC (1995) Tributyltin contamination and its impact on *Nucella lapillus* populations. Proc Roy Soc Edinb B Biol Sci 103:113–126

Bandow C, Weltje L (2012) Development of an embryo toxicity test with the pond snail *Lymnaea stagnalis* using the model substance tributyltin and common solvents. Sci Total Environ 435–436:90–95

Barroso CM, Moreira MH, Bebianno MJ (2002) Imposex, female sterility and organotin contamination of the prosobranch *Nassarius reticulatus* from the Portuguese coast. Mar Ecol Prog Ser 230:127–135

Bartlett AJ, Borgmann U, Dixon DG, Batchelor SP, Maguire RJ (2007) Comparison of toxicity and bioaccumulation of tributyltin in Hyalella azteca and five other freshwater invertebrates. Water Qual Res J Can 42:1–10

Batley GE, Scammell MS, Brockbank CI (1992) The impact of the banning of tributyltin-based antifouling paints on the Sydney rock oyster, *Saccostrea commercialis*. Sci Total Environ 122:301–314

Belcher SM, Cookman CJ, Patisaul HB, Stapleton HM (2014) *In vitro* assessment of human nuclear hormone receptor activity and cytotoxicity of the flame retardant mixture FM 550 and its triarylphosphate and brominated components. Toxicol Lett 228:93–102

Bentivegna CS, Piatkowski T (1998) Effects of tributyltin on medaka (*Oryzias latipes*) embryos at different stages of development. Aquat Toxicol 44:117–128

Birch GF, Scammell MS, Besley CH (2014) The recovery of oyster (*Saccostrea glomerata*) populations in Sydney estuary (Australia). Environ Sci Pollut Res Int 21:766–773

Birchenough AC, Evans SM, Moss C, Welch R (2002) Re-colonisation and recovery of populations of dogwhelks *Nucella lapillus* (L.) on shores formerly subject to severe TBT contamination. Mar Pollut Bull 44:652–659

Blackmore G (2000) Imposex in *Thais clavigera* (Neogastropoda) as an indicator of TBT (tributyltin) bioavailability in coastal waters of Hong Kong. J Moll Stud 66:1–8

Blomhoff R, Blomhoff HK (2006) Overview of retinoid metabolism and function. J Neurobiol 66:606–630

Boulahtouf A, Grimaldi M, Coutellec M-A, Besnard A-L, Echasseriau Y, Bourguet W, Balaguer P, Lagadic L (2015) Ligand affinity of the *Lymnaea stagnalis* estrogen and retinoid-X receptors (LsER and LsRXR): implications for detecting endocrine disruptors. 25th SETAC Europe annual meeting, Barcelona, extended abstract

Bouton D, Escriva H, de Mendonca RL, Glineur BB, Noel C, Robinson-Rechavi M, de Groot A, Cornette J, Laudet V, Pierce RJ (2005) A conserved retinoid X receptor (RXR) from the mollusk *Biomphalaria glabrata* transactivates transcription in the presence of retinoids. J Mol Endocrinol 34:567–582

Bray S, McVean EM, Nelson A, Herbert RJH, Hawkins SJ, Hudson MD (2012) The regional recovery of *Nucella lapillus* populations from marine pollution, facilitated by man-made structures. J Mar Biol Assoc UK 92:1585–1594

Bryan GW, Gibbs PE, Burt GR, Hummerstone LG (1987) The effects of tributyltin (TBT) accumulation on adult dogwhelks, *Nucella lapillus*: long term field and laboratory experiments. J Mar Biol Assoc UK 67:525–544

Bryan GW, Burt GR, Gibbs PE, Pascoe PL (1993) *Nassarius reticulatus* (Nassariidae: Gastropoda) as an indicator of tributyltin pollution before and after TBT restrictions. J Mar Biol Assoc UK 73:913–929

Campo-Paysaa F, Marlétaz F, Laudet V, Schubert M (2008) Retinoic acid signaling in development: tissue-specific functions and evolutionary origins. Genesis 46:640–656

Castro LFC, Lima D, Machado A, Melo C, Hiromori Y, Nishikawa J, Nakanishi T, Reis-Henriques MA, Santos MM (2007) Imposex induction is mediated through the retinoid X receptor signaling pathway in the neogastropod *Nucella lapillus*. Aquat Toxicol 85:57–66

Chamorro-García R, Sahu M, Abbey RJ, Laude J, Pham N, Blumberg B (2013) Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. Environ Health Perspect 121:359–366

Champ MA (2000) A review of organotin regulatory strategies, pending actions, related costs and benefits. Sci Total Environ 258:21–71

Chang X, Kobayashi T, Senthilkumaran B, Kobayashi-Kajura H, Sudhakumari CC, Nagahama Y (2005) Two types of aromatase with different encoding genes, tissue distribution and developmental expression in Nile tilapia (Oreochromis niloticus) Gen Comp Endocrinol 141:101–115

Cheshenko K, Pakdel F, Segner H, Kah O, Eggen RI (2008) Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish. Gen Comp Endocrinol 155:31–62

Choi MJ, Kim SC, Kim AN, Kwon HB, Ahn RS (2007) Effect of endocrine disruptors on the oocyte maturation and ovulation in amphibians, *Rana dybowskii*. Integr Biosci 11:1–8

Choudhury AKR (2014) Environmental impacts of the textile industry and its assessment through life cycle management. In: Muthu SS (ed) Roadmap to sustainable textiles and clothing. Environmental and social aspects of textiles and clothing supply chain. Springer, Singapore, pp 1–40

Coenen TMM, Brouwer A, Enninga IC, Koeman JH (1992) Subchronic toxicity and reproduction effects of tri-n-butyltin oxide in Japanese quail. Arch Environ Contam Toxicol 23:457–463

Coenen TMM, Enninga IC, Cave DA, Hoeven JCM (1994) Hematology and serum biochemistry of Japanese quail fed dietary tri-n-butyltin oxide during reproduction. Arch Environ Contam Toxicol 26:227–233

Colliar L, Sturm A, Leaver MJ (2011) Tributyltin is a potent inhibitor of piscine peroxisome proliferator-activated receptor alpha and beta. Comp Biochem Physiol C 153:168–173

Cooke GM (2002) Effect of organotins on human aromatase activity *in vitro*. Toxicol Lett 126:121–130

Cooke GM, Forsyth DS, Bondy GS, Tachon R, Tague B, Coady L (2008) Organotin speciation and tissue distribution in rat dams, fetuses, and neonates following oral administration of tributyltin chloride. J Toxicol Environ Health A 71:384–395

Couceiro L, Diaz J, Albaina N, Barreiro R, Irabien JA, Ruiz JM (2009) Imposex and gender-independent butyltin accumulation in the gastropod *Nassarius reticulatus* from the Cantabrian coast (N Atlantic Spain). Chemosphere 76:424–427

Decherf S, Seugnet I, Fini J-B, Clerget-Froidevaux M-S, Demeneix BA (2010) Disruption of thyroid hormone-dependent hypothalamic set-points by environmental contaminants. Mol Cell Endocrinol 323:172–182

Duft M, Schmitt C, Bachmann J, Brandelik C, Schulte-Oehlmann U, Oehlmann J (2007) Prosobranch snails as test organisms for the assessment of endocrine active chemicals—an overview and a guideline proposal for a reproduction test with the freshwater mudsnail *Potamopyrgus antipodarum*. Ecotoxicology 16:169–182

ECHA (2008) Member state committee support document for identification of Bis(tributyltin) oxide as a substance of very high concern. European Chemicals Agency, Oct 2008

Elliott JE, Harris ML, Wilson LK, Smith BD, Batchelor SP, Maguire J (2007) Butyltins, trace metals and morphological variables in surf scoter (*Melanitta perspicillata*) wintering on the south coast of British Columbia, Canada. Environ Pollut 149:114–124

European Union (EU) (2005) Common implementation strategy for the water framework directive: environmental quality standards (EQS), Substance Data Sheet Priority Substance No 30: Tributyltin compounds (TBT-ion) Final version, Brussels, Jan 2005

Faqi SA, Hilbig V, Pfeil R, Solecki R (1999) Dietary TBTO exposure to the Japanese quail: relation between exposure period and appearance of reproductive effects. Bull Environ Contam Toxicol 63:415–422

Fent K (1996) Ecotoxicology of organotin compounds. Crit Rev Toxicol 26:1–117

Fent K, Hunn J (1996) Cytotoxicity of organic environmental chemicals to fish liver cells (PLHC-1). Mar Environ Res 42:377–382

Gagné F, Blaise C, Salazar M, Salazar S, Hansen PD (2001) Evaluation of estrogenic effects of municipal effluents to the freshwater mussel *Elliptio complanata*. Comp Biochem Physiol C 128:213–225

Gibbs PE (1996) Oviduct as a sterilising effect of tributyltin (TBT)-induced imposex in *Ocenebra erinacea* (Gastropoda: Muricidae). J Moll Stud 62:403–413

Gibbs PE (2009) Long-term tributyltin (TBT)-induced sterilization of neogastropods: persistence of effects in Ocenebra erinacea over 20 years in the vicinity of Falmouth (Cornwall, UK). J Mar Biol Assoc UK 89:135–138

Gibbs PE, Bryan GW (1986) Reproductive failure in populations of the dogwhelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. J Mar Biol Assoc UK 66:767–777

Gibbs PE, Bryan GW (1996a) Reproductive failure in the gastropod *Nucella lapillus* associated with imposex caused by tributyltin pollution: a review. In: Champ MA, Seligman PF (eds) Organotin. Chapman and Hall, London, UK, pp 259–280

Gibbs PE, Bryan GW (1996b) TBT-induced imposex in neogastropod snails: masculinization to mass extinction. In: De Mora SJ (ed) Tributyltin: case study of an environmental contaminant. Cambridge University Press, New York, NY, USA, pp 212–236

Gibbs PE, Bryan GW, Pascoe PL, Burt GR (1987) The use of the dog-whelk, *Nucella lapillus,* as an indicator of tributyltin (TBT) contamination. J Mar Biol Assoc UK 67:507–523

Gibbs PE, Pascoe PL, Burt GR (1988) Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. J Mar Biol Assoc UK 68:715–731

Giusti A (2013) Impacts and mechanisms of action of endocrine disrupting chemicals on the hermaphroditic freshwater gastropod *Lymnaea stagnalis* (Linnaeus, 1758). PhD thesis, University of Liège, Belgium, 244 p

Giusti A, Joaquim-Justo C (2013) Esterification of vertebrate-like steroids in molluscs: a target of endocrine disruptors? Comp Biochem Physiol C 158:187–198

Giusti A, Barsi A, Dugué M, Collinet M, Thome JP, Joaquim-Justo C, Roig B, Lagadic L, Ducrot V (2013a) Reproductive impacts of tributyltin (TBT) and triphenyltin (TPT) in the hermaphroditic freshwater gastropod *Lymnaea stagnalis*. Environ Toxicol Chem 32:1552–1560

Giusti A, Ducrot V, Joaquim-Justo C, Lagadic L (2013b) Testosterone levels and fecundity in the hermaphroditic aquatic snail *Lymnaea stagnalis* exposed to testosterone and endocrine disruptors. Environ Toxicol Chem 32:1740–1745

Gooding MP, LeBlanc GA (2001) Biotransformation and disposition of testosterone in the eastern mud snail *Ilyanassa obsoleta*. Gen Comp Endocrinol 122:172–180

Gooding MP, Wilson VS, Folmar LC, Marcovich DT, LeBlanc GA (2003) The biocide tributyltin reduces the accumulation of testosterone as fatty acid esters in the mud snail (*Ilyanassa obsoleta*). Environ Health Perspect 111:426–430

Grimaldi M, Boulahtouf A, Delfosse V, Thouennon E, Bourguet W, Balaguer P (2015) Reporter cell lines for the characterization of the interactions between human nuclear receptors and endocrine disruptors. Front Endocrinol 6:62. https://doi.org/10.3389/fendo.2015.00062

Grün F, Watanabe H, Zamanian Z, Maeda L, Arima K, Cubacha R, Gardiner DM, Kanno J, Iguchi T, Blumberg B (2006) Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. Mol Endocrinol 20:2141–2155

Guo S, Quan L, Shi H, Barry T, Cao Q, Liu J (2010) Effects of tributyltin (TBT) on *Xenopus tropicalis* embryos at environmentally relevant concentrations. Chemosphere 79:529–533

Gutierrez-Mazariegos J, Nadendla EK, Lima D, Pierzchalski K, Jones JW, Kane M, Nishikawa JI, Hiromori Y, Nakanishi T, Santos MM, Castro LFC, Bourguet W, Schubert M, Laudet V (2014) A mollusk retinoic acid receptor (RAR) ortholog sheds light on the evolution of ligand binding. Endocrinology 155:4275–4286

Hahn T, Schulz R (2002) Ecdysteroid synthesis and imaginal disc development in the midge *Chironomus riparius* as biomarkers for endocrine effects of tributyltin. Environ Toxicol Chem 21:1052–1057

Hano T, Oshima Y, Kim SG, Satone H, Oba Y, Kitano T, Inoue S, Shimasaki Y, Honjo T (2007) Tributyltin causes abnormal development in embryos of medaka, *Oryzias latipes*. Chemosphere 69:927–933

Harazono A, Ema M, Ogawa Y (1996) Pre-implantation embryonic loss induced by tributyltin chloride in rats. Toxicol Lett 89:185–190

Harazono A, Ema M, Ogawa Y (1998) Evaluation of early embryonic loss induced by Tributyltin chloride in rats: phase- and dose-dependent antifertility effects. Arch Environ Contam Toxicol 34:94–99

Harding MJC, Rodger GK, Davies IM, Moore JJ (1997) Partial recovery of the dogwhelk (*Nucella lapillus*) in Sullom Voe, Shetland from tributyltin contaminations. Mar Environ Res 44:285–304

Haubruge E, Petit F, Gage MJ (2000) Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyltin and bisphenol A. Proc Roy Soc Lond B 67:2333–2337

Hawkins SJ, Proud SV, Spence SK, Southward AJ (1994) From the individual to the community and beyond: water quality, stress indicators and key species in coastal waters. In: Sutcliffe DW (ed) Water quality & stress indicators in marine and freshwater systems: linking levels of organisation. Ambleside, UK, Freshwater Biological Association, pp 35–62

Heidrich DD, Steckelbroeck S, Klingmuller D (2001) Inhibition of human cytochrome P450 aromatase activity by butyltins. Steroids 66:763–769

Ho KKY, Zhou G-J, EGB X, Wang X, Leung KMY (2016) Long-term spatio-temporal trends of organotin contaminations in the marine environment of Hong Kong. PLoS One 11(5): e0155632

Holbech H, Kinnberg K, Petersen GI, Jackson P, Hylland K, Norrgren L, Bjerregaard P (2006) Detection of endocrine disrupters: evaluation of a fish sexual development test (FSDT). Comp Biochem Physiol C 144:57–66

Horiguchi T, Shiraishi H, Shimizu M, Morita M (1994) Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. J Mar Biol Assoc UK 74:651–669

Huang Y, Zhu L, Liu G (2006) The effects of bis(tributyltin) oxide on the development, reproduction and sex ratio of calanoid copepod *Pseudodiaptomus marinus*. Estuar Coast Shelf Sci 69:147–152

Huang Y, Zhu LY, Qiu XC, Zhang TW (2010) Effect of bis(tributyltin) oxide on reproduction and population growth rate of calanoid copepod *Schmackeria poplesia*. Chinese J Oceanol Limnol 28:280–287

Iguchi T, Katsu Y (2008) Commonality in signaling of endocrine disruption from snail to human. Bioscience 58:1061–1067

Inoue S, Oshima Y, Nagai K, Yamamoto T, Go J, Kai N, Honjo T (2004) Effect of maternal exposure to tributyltin on reproduction of the pearl oyster (Pinctada fucata martensii). Environ Toxicol Chem 23:1276–1281

Inoue S, Oshima Y, Usuki H, Hamaguchi M, Hanamura Y, Kai N, Shimasaki Y, Honjo T (2006) Effects of tributyltin maternal and/or waterborne exposure on the embryonic development of the Manila clam, *Ruditapes philippinarum*. Chemosphere 63:881–888

Janer G, Lavado R, Thibaut R, Porte C (2004) Effects of 17b-estradiol exposure in the mussel *Mytilus galloprovincialis*. Mar Environ Res 58:443–446

Jordão R, Casas J, Fabrias G, Campos B, Piña B, Lemos MF, Soares AM, Tauler R, Barata C (2015) Obesogens beyond vertebrates: lipid perturbation by tributyltin in the crustacean *Daphnia magna*. Environ Health Perspect 123:813–819

Kanayama T, Kobayashi N, Mamiya S, Nakanishi T, Nishikawa J (2005) Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor γ/retinoid-X receptor pathway. Mol Pharmacol 67:766–774

Kanda A, Takahashi T, Satake H, Minakata H (2006) Molecular and functional characterization of a novel gonadotropin-releasing-hormone receptor isolated from the common octopus (*Octopus vulgaris*). Biochem J 395:125–135

Kaur S, Jobling S, Jones CS, Noble LR, Routledge EJ, Lockyer AE (2015) The nuclear receptors of Biomphalaria glabrata and Lottia gigantea: implications for developing new model organisms. PLoS One 10(4)

Kazeto Y, Ijiri S, Place AR, Zohar Y, Trant JM (2001) The 5′-flanking regions of CYP19A1 and CYP19A2 in zebrafish. Biochem Biophys Res Comm 288:503–508

Kim NS, Hong SH, Yim UH, Shin K-H, Shim WJ (2014) Temporal changes in TBT pollution in water, sediment, and oyster from Jinhae Bay after the total ban in South Korea. Mar Pollut Bull 86:547–554

Kinnberg K, Holbech H, Petersen GI, Bjerregaard P (2007) Effects of the fungicide prochloraz on the sexual development of zebrafish (*Danio rerio*). Comp Biochem Physiol C 145:165–170

Kirchner S, Kieu T, Chow C, Casey S, Blumberg B (2010) Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. Mol Endocrinol 24:526–539

Kishida M, Nakao R, Novillo A, Callard IP, Osada M (2005) Molecular cloning and expression analysis of cDNA fragments related to estrogen receptor from blue mussel, *Mytilus edulis*. Proc Jpn Soc Comp Endocrinol 20:75

Kishta O, Adeeko A, Li D, Luu T, Brawer JR, Morales C, Hermo L, Robaire B, Hales BF, Barthelemy J, Cyr DG, Trasler JM (2007) In utero exposure to tributyltin chloride differentially alters male and female fetal gonad morphology and gene expression profiles in the Sprague–Dawley rat. Reprod Toxicol 23:1–11

Kortner TM, Pavlikova N, Arukwe A (2010) Effects of tributyltin on salmon interrenal CYP11 beta, steroidogenic factor-1 and glucocorticoid receptor transcripts in the presence and absence of second messenger activator, forskolin. Mar Environ Res 69:S56–S58

Kusk KO, Petersen S (1997) Acute and chronic toxicity of tributyltin and linear alkylbenzene sulfonate to the marine copepod *Acartia tonsa*. Environ Toxicol Chem 16:1629–1633

Laflamme L, Hamann G, Messier N, Maltais S, Langlois M-F (2002) RXR acts as a coregulator in the regulation of genes of the hypothalamo-pituitary axis by thyroid hormone receptors. J Mol Endocrinol 29:61–72

Lagerström M, Strand J, Eklund Y (2016) Total tin and organotin speciation in historic layers of antifouling paint on leisure boat hulls. Environ Pollut 220(B):1333–1341
LeBlanc GA, Gooding MP, Sternberg RM (2005) Testosterone-fatty acid esterification: a unique target for the endocrine toxicity of tributyltin to gastropods. Integr Comp Biol 45:81–87
Leung KMY, Kwong RPY, Ng WC, Horiguchi T, Qiu JW, Yang RQ, Song MY, Jiang GB, Zheng GJ, Lam PKS (2006) Ecological risk assessments of endocrine disrupting organotin compounds using marine neogastropods in Hong Kong. Chemosphere 65:922–938
Leung KMY, Grist EPM, Morley NJ, Morritt D, Crane M (2007) Chronic toxicity of tributyltin to development and reproduction of the European freshwater snail *Lymnaea stagnalis* (L.) Chemosphere 66:1358–1366
Lewis JA, Baran IJ, Carey JM, Fletcher LE (2010) A contaminant in decline: long-term TBT monitoring at a naval base in Western Australia. Aust J Ecotoxicol 16:17–34
Lilley TM, Ruokolainen L, Pikkarainen A, Laine VN, Kilpimaa J, Rantala MJ, Nikinmaa M (2012) Impact of tributyltin on immune response and life history traits of *Chironomus riparius*: single and multigeneration effects and recovery from pollution. Environ Sci Technol 46:7382–7389
Lima D, Reis-Henriques MA, Silva R, Santos AI, Castro LFC, Santos MM (2011) Tributyltin-induced imposex in marine gastropods involves tissue-specific modulation of the retinoid X receptor. Aquat Toxicol 101:221–227
Lima D, Castro LFC, Coelho I, Lacerda R, Gesto M, Soares J, André A, Capela R, Torres T, Carvalho AP, Santos MM (2015) Effects of tributyltin and other retinoid receptor agonists in reproductive-related endpoints in the zebrafish (*Danio rerio*). J Toxicol Environ Health A 78:747–760
Maack G, Segner H (2003) Morphological development of the gonads in zebrafish. J Fish Biol 62:895–906
le Maire A, Grimaldi M, Roecklin D, Dagnino S, Vivat-Hannah V, Balaguer P, Bourguet W (2009) Activation of RXR-PPAR heterodimers by organotin environmental endocrine disruptors. EMBO Rep 10:367–373
Matthiessen P, Gibbs PE (1998) Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in molluscs. Environ Toxicol Chem 17:37–43
Matthiessen P, Weltje L (2015) A review of the effects of azole compounds in fish and their possible involvement in masculinization of wild fish populations. Crit Rev Toxicol 45:453–467
McAllister BG, Kime DE (2003) Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish (*Danio rerio*). Aquat Toxicol 65:309–316
McCarty LS, Landrum PF, Luoma SN, Meador JP, Merten AA, Shephard BK, van Wezel AP (2011) Advancing environmental toxicology through chemical dosimetry: external exposures versus tissue residues. Integr Environ Assess Manag 7:7–27
McElroy AE, Barron MG, Beckvar N, Kane Driscoll SB, Meador JP, Parkerton TF, Preuss TG, Steevens JA (2011) A review of the tissue residue approach for organic and organometallic compounds in aquatic organisms. Integr Environ Assess Manag 7:50–74
McGinnis CL, Crivello JF (2011) Elucidating the mechanism of action of tributyltin (TBT) in zebrafish. Aquat Toxicol 103:25–31
McGinnis CL, Encarnacao PC, Crivello JF (2012) Dibutyltin (DBT): an endocrine disrupter in zebrafish. J Exp Mar Biol Ecol 430–431:43–47
McVey MJ, Cooke GM (2003) Inhibition of rat testis microsomal 3β-hydroxysteroid dehydrogenase activity by tributyltin. J Steroid Biochem Mol Biol 86:99–105
Meador JP (1997) Comparative toxicokinetics of tributyltin in five marine species and its utility in predicting bioaccumulation and toxicity. Aquat Toxicol 37:307–326
Meador JP (2000) Predicting the fate and effects of tributyltin in marine systems. Rev Environ Contam Toxicol 166:1–48

Meador JP (2006) Rationale and procedures for using the tissue-residue approach for toxicity assessment and determination of tissue, water, and sediment quality guidelines for aquatic organisms. Hum Ecol Risk Assess 12:1018–1073

Meador JP (2011) Organotins in aquatic biota: occurrence in tissue and toxicological significance. In: Beyer WN, Meador JP (eds) Environmental contaminants in biota: interpreting tissue concentrations. Taylor and Francis, Boca Raton, FL, pp 255–284

Meador JP, McCarty LS, Escher BI, Adams WJ (2008) The tissue-residue approach for toxicity assessment: concepts, issues, application, and recommendations. J Environ Monit 10:1486–1498

Meador JP, Sommers FC, Cooper K, Yanagida G (2011) Tributyltin and the obesogen metabolic syndrome in a salmonid. Environ Res 111:50–56

Mengeling BJ, Murk AJ, Furlow JD (2016) Trialkyltin retinoid-X receptor agonists selectively potentiate thyroid hormone induced programs of Xenopus laevis metamorphosis. Endocrinology 157:2712–2723

Mizukawa H, Takahashi S, Nakayama K, Sudo A, Tanabe S (2009) Contamination and accumulation feature of organotin compounds in common cormorants (*Phalacrocorax carbo*) from Lake Biwa, Japan. In: Obayashi Y, Isobe T, Subramanian A, Suzuki S, Tanabe S (eds) Interdisciplinary studies on environmental chemistry — environmental research in Asia. TERRAPUB, pp 153–161

Mochida K, Ito K, Kono K, Onduka T, Kakuno A, Fujii K (2007) Molecular and histological evaluation of tributyltin toxicity on spermatogenesis in a marine fish, the mummichog (*Fundulus heteroclitus*). Aquat Toxicol 83:73–83

Mochida K, Ito K, Kono K, Onduka T, Kakuno A, Fujii K (2010) Effect of tributyltin oxide exposure on the F-0 and F-1 generations of a marine teleost, the mummichog *Fundulus heteroclitus*. Fish Sci 76:333–341

Morales M, Martinez-Paz P, Ozaez I, Martinez-Guitarte JL, Morcillo G (2013) DNA damage and transcriptional changes induced tributyltin (TBT) after short *in vivo* exposures of *Chironomus riparius* (Diptera) larvae. Comp Biochem Physiol C 158:57–63

Morcillo Y, Janer G, O'Hara SCM, Livingstone DR, Porte C (2004) Interaction of tributyltin with hepatic cytochrome P450 and uridine diphosphate-glucoronosyl transferase systems of fish: in vitro studies. Environ Toxicol Chem 23:990–996

Mortensen AS, Arukwe A (2007) Modulation of xenobiotic biotransformation system and hormonal responses in Atlantic salmon (*Salmo salar*) after exposure to tributyltin (TBT). Comp Biochem Physiol C 145:431–441

Mortensen AS, Arukwe A (2009) Effects of tributyltin (TBT) on *in vitro* hormonal and biotransformation responses in Atlantic salmon (*Salmo salar*). J Toxicol Environ Health A 72:209–218

Morthorst JE, Holbech H, Jeppesen M, Kinnberg KL, Pedersen KL, Bjerregaard P (2014) Evaluation of yolk protein levels as estrogenic biomarker in bivalves; comparison of the alkali-labile phosphate method (ALP) and a species-specific immunoassay (ELISA). Comp Biochem Physiol C 166:88–95

Nakanishi T, Kohroki J, Suzuki S, Ishizaki J, Hiromori Y, Takasuga S, Itoh N, Watanabe Y, Utoguchi N, Tanaka K (2002) Trialkyltin compounds enhance human CG secretion and aromatase activity in human placental choriocarcinoma cells. J Clin Endocrinol Metab 87:2830–2837

Nakanishi T, Nishikawa J, Hiromori Y, Yokoyama H, Koyanagi M, Takasuga S, Ishizaki J, Watanabe M, Isa S, Utoguchi N, Itoh N, Kohno Y, Nishihara T, Tanaka K (2005) Trialkyltin compounds bind retinoid-X receptor to alter human placental endocrine functions. Mol Endocrinol 19:2502–2516

Nakanishi I, Nishikawa J, Tanaka K (2006) Molecular targets of organotin compounds in endocrine disruption: do organotin compounds function as aromatase inhibitors in mammals? Environ Sci 13:89–100.

Nakayama K, Oshima Y, Yamaguchi T, Tsuruda Y, Kang IJ, Kobayashi M, Imada N, Honjo T (2004) Fertilization succes and sexual behavior in male medaka, *Oryzias latipes*, exposed to tributyltin. Chemosphere 55:1331–1337

Nakayama K, Oshima Y, Nagafuchi K, Hano T, Shimasaki Y, Honjo T (2005) Early-life stage toxicity in offspring from exposed parent Medaka, *Oryzias latipes*, to mixtures of tributyltin and polychlorinated biphenyls. Environ Toxicol Chem 24:591–596

Neuwoehner J, Junghans M, Koller M, Escher BI (2008) QSAR analysis and specific endpoints for classifying the physiological modes of action of biocides in synchronous green algae. Aquat Toxicol 90:8–18

Nicolaus EEM, Barry J (2015) Imposex in the dogwhelk (*Nucella lapillus*): 22-year monitoring around England and Wales. Environ Monit Assess 187:736

Nishikawa J, Mamiya S, Kanayama T, Nishikawa T, Shiraishi F, Horiguchi T (2004) Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods. Environ Sci Technol 38:6271–6276

Nunez SB, Medin JA, Braissant O, Kemp L, Wahli W, Ozato K, Segars JH (1997) Retinoid X receptor and peroxisome proliferatoractivated receptor activate an estrogen responsive gene independent of the estrogen receptor. Mol Cell Endocrinol 127:27–40

Oberdorster E, McClellan-Green P (2002) Mechanisms of imposex induction in the mud snail, *Ilyanassa obsoleta*: TBT as a neurotoxin and aromatase inhibitor. Mar Environ Res 54:715–718

Oberdorster E, Rittschof D, LeBlanc GA (1998) Alteration of [C-14]-testosterone metabolism after chronic exposure of *Daphnia magna* to tributyltin. Arch Environ Contam Toxicol 34:21–25

OECD (2007) SIDS initial assessment profile for SIAM 24, 19–20 April, 2007. Tributyltin chloride, CAS no. 1461-22-9. http://webnet.oecd.org/Hpv/ui/handler.axd?id=738dc513-4ec8-4d55-ac11-4e1ff79123b8

OECD (2010) Detailed review paper (DRP) on molluscs life-cycle toxicity testing. OECD Environment, Health and Safety Publications, Series on Testing and Assessment, no. 121, pp 182

OECD (2012a) Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption. Series on testing and assessment, no. 150, pp 524

OECD (2012b) No 142 validation report (phase 2) for the fish sexual development test for the detection of endocrine active substances. Paris, France, OECD ENV/JM/MONO(2011)23/REV1

Oehlmann J, Di Benedetto P, Tillmann M, Duft M, Oetken M, Schulte-Oehlmann U (2007) Endocrine disruption in prosobranch molluscs: evidence and ecological relevance. Ecotoxicology 16:29–43

Ogata R, Omura M, Shimasaki Y, Kubo K, Oshima Y, Aou S, Inoue N (2001) Two-generation reproductive toxicity study of tributyltin chloride in female rats. J Toxicol Environ Health 63:127–144

Ohhira S, Watanabe M, Matsui H (2003) Metabolism of tributyltin and triphenyltin by rat, hamster and human hepatic microsomes. Arch Toxicol 77:138–144

Ohhira S, Enomoto M, Matsui H (2006a) In vitro metabolism of tributyltin and triphenyltin by human cytochrome P-450 isoforms. Toxicology 228:171–177

Ohhira S, Enomoto M, Matsui H (2006b) Sex difference in the principal cytochrome P-450 for tributyltin metabolism in rats. Toxicol Appl Pharmacol 210:32–38

Ohji M, Arai T, Miyazaki N (2003a) Biological effects of tributyltin exposure on the caprellid amphipod, *Caprella danilevskii*. J Mar Biol Assoc UK 83:111–117

Ohji M, Arai T, Miyazaki N (2003b) Chronic effects of tributyltin on the caprellid amphipod *Caprella danilevskii*. Mar Pollut Bull 46:1263–1272

Ohji M, Arai T, Miyazaki N (2006) Transfer of tributyltin from parental female to offspring in the viviparous surfperch *Ditrema temmincki*. Mar Ecol Prog Ser 307:307–310

Omura M, Ogata R, Kubo K, Shimasaki Y, Aou S, Oshima Y, Tanaka A, Hirata M, Makita Y, Inoue N (2001) Two-generation reproductive toxicity study of tributyltin chloride in male rats. Toxicol Sci 64:224–232

Örn S, Yamani S, Norrgren L (2006) Comparison of vitellogenin induction, sex ratio, and gonad morphology between zebrafish and Japanese medaka after exposure to 17α-ethinylestradiol and 17β-trenbolone. Arch Environ Contam Toxicol 51:237–243

Ouadah-Boussouf N, Babin PJ (2016) Pharmacological evaluation of the mechanisms involved in increased adiposity in zebrafish triggered by the environmental contaminant tributyltin. Toxicol Appl Pharmacol 294:32–42

Parrott JL, Bjerregaard P, Brugger KE, Gray Jr LE, Iguchi T, Kadlec SM, Weltje L, Wheeler JR (2017) Uncertainties in biological responses that influence hazard and risk approaches to the regulation of endocrine active substances. Integr Environ Assess Manag 13:293–301

Pascoal S, Carvalho G, Vasieva O, Hughes R, Cossins A, Fang Y-X, Ashelford K, Olohan L, Barroso C, Mendo S, Creer S (2013) Transcriptomics and *in vivo* tests reveal novel mechanisms underlying endocrine disruption in an ecological sentinel, *Nucella lapillus*. Mol Ecol 22:1589–1608

Pavlikova N, Arukwe A (2011) Immune-regulatory transcriptional responses in multiple organs of Atlantic salmon after tributyltin exposure, alone or in combination with forskolin. J Toxicol Environ Health A 74:478–493

Pavlikova N, Kortner TM, Arukwe A (2010) Peroxisome proliferator-activated receptors, estrogenic responses and biotransformation system in the liver of salmon exposed to tributyltin and second messenger activator. Aquat Toxicol 99:176–185

Pereira ML, Eppler E, Thorpe KL, Wheeler JR, Burkhardt-Holm P (2011a) Molecular and cellular effects of chemicals disrupting steroidogenesis during early ovarian development of brown trout (*Salmo trutta fario*). Environ Toxicol 29:199–206

Pereira ML, Wheeler JR, Thorpe KL, Burkhardt-Holm P (2011b) Development of an *ex vivo* brown trout (*Salmo trutta fario*) gonad culture for assessing chemical effects on steroidogenesis. Aquat Toxicol 101:500–511

Rodney SI, Moore DTR (2008) SSD Master, Ver 2.0: determination of hazardous concentrations with species sensitivity distributions. Intrinsik, Toronto, ON, Canada

Saitoh K, Nagai F, Aoki N (2001a) Several environmental pollutants have binding affinities for both androgen receptor and estrogen receptor alpha. J Health Sci 47:495–501

Saitoh M, Yanase T, Morinaga H, Tanabe M, Y-M M, Nishi Y, Nomura M, Okabe T, Goto K, Takayanagi R, Nawata H (2001b) Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN. Biochem Biophys Res Commun 289:198–204

Santos MM, Micael J, Carvalho AP, Morabito R, Booy P, Massanisso P, Lamoree M, Reis-Henriques MA (2006) Estrogens counteract the masculinizing effect of tributyltin in zebrafish. Comp Biochem Physiol C 142:151–155

Schlatterer B, Coenen TM, Ebert E, Grau R, Hilbig V, Munk R (1993) Effects of bis(tri-n-butyltin) oxide in Japanese quail exposed during egg laying period: an interlaboratory comparison study. Arch Environ Contam Toxicol 24:440–448

Schwarz TI (2015) The origin of vertebrate steroids in molluscs: uptake, metabolism and depuration studies in the common mussel. PhD thesis, University of Glasgow/Cefas

Schwarz TI, Katsiadaki I, Maskrey BH, Scott AP (2017a) Mussels (*Mytilus* spp.) display an ability for rapid and high capacity uptake of the vertebrate steroid, estradiol-17β from water. J Steroid Biochem Mol Biol 165(B):407–420

Schwarz TI, Katsiadaki I, Maskrey BH, Scott AP (2017b) Rapid uptake, biotransformation, esterification and lack of depuration of testosterone and its metabolites by the common mussel, *Mytilus* spp. J Steroid Biochem Mol Biol 171:54–65

Scott AP (2012) Do mollusks use vertebrate sex steroids as reproductive hormones? Part I: critical appraisal of the evidence for the presence, biosynthesis and uptake of steroids. Steroids 77:1450–1468

Scott AP (2013) Do mollusks use vertebrate sex steroids as reproductive hormones? Part II. Critical review of the evidence that steroids have biological effects. Steroids 78:268–281
Sharan S, Nikhil K, Roy P (2013) Effects of low dose treatment of tributyltin on the regulation of estrogen receptor functions in MCF-7 cells. Toxicol Appl Pharmacol 269:176–186
Sharan S, Nikhil K, Roy P (2014) Disruption of thyroid hormone functions by low dose exposure of tributyltin: an *in vitro* and *in vivo* approach. Gen Comp Endocrinol 206:155–165
Shi H, Zhu P, Guo S (2014) Effects of tributyltin on metamorphosis and gonadal differentiation of *Xenopus laevis* at environmentally relevant concentrations. Toxicol Ind Health 30:297–303
Shimasaki Y, Kitano T, Oshima Y, Inoue S, Imada N, Honjo T (2003) Tributyltin causes masculinization in fish. Environ Toxicol Chem 22:141–144
Shimasaki Y, Oshima Y, Inoue S, Inoue Y, Kang IJ, Nakayama K, Imoto H, Honjo T (2006) Effect of tributyltin on reproduction in Japanese whiting, *Sillago japonica*. Mar Environ Res 62: S245–S248
Si J, Wu X, Wan C, Zeng T, Zhang M, Xie K, Li J (2011) Peripubertal exposure to low doses of tributyltin chloride affects the homeostasis of serum T, E2, LH, and body weight of male mice. Environ Toxicol 26:307–314
Si J, Han X, Zhang F, Xin Q, An L, Li G, Li C (2012) Perinatal exposure to low doses of tributyltin chloride advances puberty and affects patterns of estrous cyclicity in female mice. Environ Toxicol 27:662–670
Si J, Li P, Xin Q, Li X, An L, Li J (2013) Perinatal exposure to low doses of tributyltin chloride reduces sperm count and quality in mice. Environ Toxicol 30:44–52
Silva PV, Silva ARR, Mendo S, Loureiro S (2014) Toxicity of tributyltin (TBT) to terrestrial organisms and its species sensitivity distribution. Sci Total Environ 466–467:1037–1046
Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Corbin CJ, Mendelson CR (1993) Tissue-specific promoters regulate aromatase cytochrome P450 expression. Clin Chem 39:317–324
Smith BS (1981) Male characteristics on female mud snails caused by antifouling bottom paints. J Appl Toxicol 1:22–25
Spence SK, Bryan GW, Gibbs PE, Masters D, Morris L, Hawkins SJ (1990) Effects of TBT contaminations on *Nucella* populations. Funct Ecol 4:425–432
Strand J, Jorgensen A, Tairova Z (2009) TBT pollution and effects in molluscs at US Virgin Islands, Caribbean Sea. Environ Int 35:707–711
Takahashi H (1977) Juvenile hermaphroditism in the zebrafish, *Brachydanio rerio*. Bull Fac Fish Hokkaido Univ 28:57–65
Tian H, Wu P, Wang W, SG R (2015) Disruptions in aromatase expression in the brain, reproductive behavior, and secondary sexual characteristics in male guppies (*Poecilia reticulata*) induced by tributyltin. Aquat Toxicol 162:117–125
Tingaud-Sequeira A, Ouadah N, Babin PJ (2011) Zebrafish obesogenic test: a tool for screening molecules that target adiposity. J Lipid Res 52:1765–1772
Tonk EC, Pennings JL, Piersma AH (2015) An adverse outcome pathway framework for neural tube and axial defects mediated by modulation of retinoic acid homeostasis. Reprod Toxicol 55:104–113
Urishitani H, Katsu Y, Ohta Y, Shiraishi H, Iguchi T, Horiguchi T (2013) Cloning and characterization of the retinoic acid receptor-like protein in the rock shell, *Thais clavigera*. Aquat Toxicol 142-143:203–213
US EPA (1988) Recommendations for and documentation of biological values for use in risk assessment. EPA/600/6-87/008, Feb 1988
US EPA (2003) Ambient aquatic life water quality criteria for Tributyltin (TBT)—final. United States Environmental Protection Agency, Office of Water. EPA 822-R-03-031, Dec 2003
US EPA (2008) Ecological hazard and environmental risk assessment for Tributyltin-containing compounds (TBT). United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Jan 2008
van Vlaardingen P, Traas TP, Aldenberg T, Wintersen A (2014) ETX 2.1 software. Normal distribution based hazardous concentration and fraction affected. RIVM, The Netherlands

Varella Motta da Costa B, Yogui GT, Souza-Santos LP (2014) Acute toxicity of tributyltin on the marine copepod Tisbe biminiensis. Braz J Oceanogr 62:65–69

Vinggaard AM, Hnida C, Breinholt V, Larsen JC (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. Toxicol In Vitro 14:227–234

Vogeler S, Galloway TS, Lyons BP, Bean TP (2014) The nuclear receptor gene family in the Pacific oyster, *Crassostrea gigas*, contains a novel subfamily group. BMC Genomics 15:369

Wang YH, Wang G, LeBlanc GA (2007) Cloning and characterization of the retinoid X receptor from a primitive crustacean *Daphnia magna*. Gen Comp Endocrinol 150:309–318

Yang J, Oshima Y, Sei I, Miyazaki N (2009) Metabolism of tributyltin and triphenyltin by Dall's porpoise hepatic microsomes. Chemosphere 76:1013–1015

Yanik SC, Baker AH, Mann KK, Schlezinger JJ (2011) Organotins are potent activators of $PPAR_{\gamma}$ and adipocyte differentiation in bone marrow multipotent mesenchymal stromal cells. Toxicol Sci 122:476–488

Zhang JL, Zuo ZH, Chen YX, Zhao Y, Hu S, Wang CG (2007) Effect of tributyltin on the development of ovary in female cuvier (*Sebastiscus marmoratus*). Aquat Toxicol 83:174–179

Zhang JL, Zuo ZH, He CY, Cai JL, Wang YQ, Chen YX, Wang CG (2009a) Effect of tributyltin on testicular development in *Sebastiscus marmoratus* and the mechanisms involved. Environ Toxicol Chem 28:1528–1535

Zhang JL, Zuo ZH, He CY, Wu D, Chen YX, Wang CG (2009b) Inhibition of thyroidal status related to depression of testicular development in *Sebastiscus marmoratus* exposed to tributyltin. Aquat Toxicol 94:62–67

Zhang JL, Zuo ZH, Wang YQ, Yu A, Chen YX, Wang CG (2011) Tributyltin chloride results in dorsal curvature in embryo development of *Sebastiscus marmoratus* via apoptosis pathway. Chemosphere 82:437–442

Zhang JL, Zuo ZH, Xiong JL, Sun P, Chen YX, Wang CG (2013a) Tributyltin exposure causes lipotoxicity responses in the ovaries of rockfish, *Sebastiscus marmoratus*. Chemosphere 90:1294–1299

Zhang JL, Zuo ZH, Zhu WW, Sun P, Wang CG (2013b) Sex-different effects of tributyltin on brain aromatase, estrogen receptor and retinoid X receptor gene expression in rockfish (*Sebastiscus marmoratus*). Mar Environ Res 90:113–118

Zhang J, Sun p YF, Kong T, Zhang R (2016) Tributyltin disrupts feeding and energy metabolism in the goldfish (Carassius auratus). Chemosphere 152:221–228

Zuo ZH, Cai JL, Wang XL, Li BW, Wang CG, Chen YX (2009) Acute administration of tributyltin and trimethyltin modulate glutamate and N-methyl-D-aspartate receptor signaling pathway in *Sebastiscus marmoratus*. Aquat Toxicol 92:44–49

Zuo Z, Wu T, Lin M, Zhang S, Yan F, Yang Z, Wang Y, Wang C (2014) Chronic exposure to tributyltin chloride induces pancreatic islet cell apoptosis and disrupts glucose homeostasis in male mice. Environ Sci Technol 48:5179–5186

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Article

Emerging Contaminants and New POPs (PFAS and HBCDD) in Endangered Southern Resident and Bigg's (Transient) Killer Whales (*Orcinus orca*): *In Utero* Maternal Transfer and Pollution Management Implications

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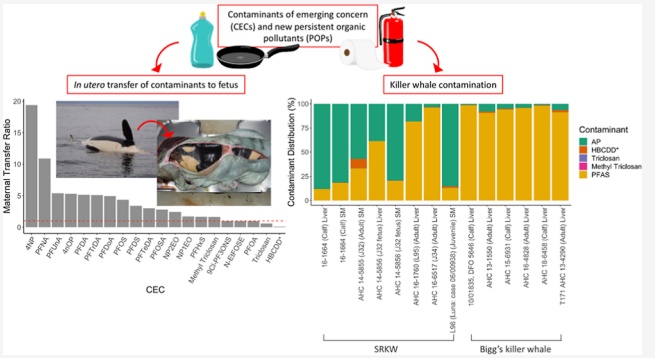
ABSTRACT: Killer whales (*Orcinus orca*) have been deemed one of the most contaminated cetacean species in the world. However, concentrations and potential health implications of selected 'contaminants of emerging concern' (CECs) and new persistent organic pollutants (POPs) in endangered Southern Resident and threatened Bigg's (Transient) killer whales in the Northeastern Pacific (NEP) have not yet been documented. Here, we quantify CECs [alkylphenols (APs), triclosan, methyl triclosan, and per- and polyfluoroalkyl substances (PFAS)] and new POPs [hexabromocyclododecane (HBCCD), PFOS, PFOA, and PFHxS] in skeletal muscle and liver samples of these sentinel species and investigate *in utero* transfer of these contaminants. Samples were collected from necropsied individuals from 2006 to 2018 and analyzed by LC−MS/MS or HRBC/HRMS. AP and PFAS contaminants were the most prevalent compounds; 4-nonylphenol (4NP) was the predominant AP (median 40.84 ng/g ww), and interestingly, 7:3-fluorotelomer carboxylic acid (7:3 FTCA) was the primary PFAS (median 66.35 ng/g ww). Maternal transfer ratios indicated 4NP as the most transferred contaminant from the dam to the fetus, with maternal transfer rates as high as 95.1%. Although too few killer whales have been screened for CECs and new POPs to infer the magnitude of contamination impact, these results raise concerns regarding pathological implications and potential impacts on fetal development and production of a viable neonate. This study outlines CEC and new POP concentrations in killer whales of the NEP and provides scientifically derived evidence to support and inform regulation to mitigate pollutant sources and contamination of Southern Resident killer whale critical habitat and other marine ecosystems.



KEYWORDS: *marine ecotoxicology, contaminants of emerging concern, endangered killer whales, alkylphenols, per- and polyfluoroalkyl substances, maternal transfer*

INTRODUCTION

The ubiquity of anthropogenic chemical contaminants and ocean pollution is a significant concern to human health, and marine ecosystems and biodiversity.[1−3] Bioaccumulative and toxic pollutants such as PFOS, PFOA, PFHxS [all classified as per- and polyfluoroalkyl substances (PFAS)], and hexabromocyclododecane (HBCDD) have recently been added to the Stockholm convention on persistent organic pollutants (POPs).[4] Although regulated under this organization, such new POPs can still be manufactured in several countries and can be produced as byproducts of certain chemicals;[4] for example, HBCDD is still used as a flame retardant in polystyrene materials. Another group of chemicals termed "contaminants of emerging concern" (CECs) including alkylphenols (AP), triclosan, methyl triclosan, and other PFAS compounds have been detected in the marine environment; however, they are not well understood in this context and, consequently, are not well regulated. Both CECs and new POPs can be found in everyday products such as pesticides, surfactants, flame retardants, antibacterial consumer items, and water-repellant materials.[5−8] They may be inefficiently removed in wastewater treatment plants and may be poorly monitored in industrial, agricultural, and residential leaks and runoffs.[9,10]

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Table 1. Biometrics and Descriptions of Analyzed Tissue in the 12 Stranded Killer Whales (*O. orca*) Collected in British Columbia, Canada, 2006−2018

killer whale identification (ID)	recovery date	location	age category	age estimate (year)	sex	ecotype	sample(s) analyzed (SM = skeletal muscle)	carcass condition code	body condition index (BCI)	percent (%) lipid	pathological findings (Raverty et al.;[38] unpublished data)	total contaminant concentration (ng/g in ww, lw)	dominant contaminant
L98 (Luna: case 06/00938)	2006-03-10	Nootka Sound, Gold River, BC	Juvenile	7	Male	SRKW	SM	2	NA	7.76	Trauma (boat strike).	136.3, 1756.5	4NP
10/01835, DFO 5646	2010-05-04	Sooke, BC	Neonate	0.1[a]	Male	Bigg's	Liver	3	NA	3.65	Failure of passive transfer of maternal antibodies/live strand.	554.3, 15185	7:3 FTCA
AHC 13−1550	2013-04-13	Carmanah Beach, BC	Adult	NA	Female	Bigg's	Liver	4	NA	13.4	Autolysis, possible subcutaneous hematoma around blowhole.	186.7, 1393.2	7:3 FTCA
T171 AHC 13−4290	2013-10-18	Prince Rupert, BC	Adult	29	Female	Bigg's	Liver	Late 3	0.56	10.4	Vertebral bridging spondylosis with emaciation.	1028.3, 9887.9	7:3 FTCA
AHC 14−5855 (J32 Mother)	2014-12-06	Comox, BC	Adult	18	Female	SRKW	SM	3	0.65	2.76	Dystocia and endometrial perforation.	25.6, 927.8	NP1EO
AHC 14−5856 (J32 Fetus)	2014-12-06	Comox, BC	Fetus	NA	Female	SRKW	SM + Liver	3	0.73	5.9 (SM) + 4.48 (Liver)	Fetal breech presentation.	125.7, 2130.2 (SM) + 306.5, 6840.7 (Liver)	4NP
AHC 15−6931	2015-12-25	Tofino, BC	Neonate	0.1[a]	Female	Bigg's	Liver	3	0.05	3.83	Presumptive metabolic derangements, hypoglycemia, possible dystocia.	516.9, 13495.1	7:3 FTCA
16−1664	2016-03-25	Sooke	Neonate	0.1[a]	Female	SRKW	SM + Liver	3	NA	1.3 (SM) + 16.3(Liver)	Trauma, inadvertent or deliberate aggression from pod mates, con-specifics, or mother (mismothering).	197.2, 15171.9 (SM) + 3799.0, 23306.7 (Liver)	4NP
AHC 16−1760 (L95)	2016-03-31	Esperanza Inlet, BC	Adult	20	Male	SRKW	Liver	4	NA	11.3	Mucormycosis secondary to satellite tag implant	423.1, 3744	PFOSA
AHC 16−4828	2016-09-15	Pachena Bay, BC	Adult	34	Male	Bigg's	Liver	3	0.6	9.36	Trauma, possible vessel strike and aggression by conspecifics.	476.5, 5091	7:3 FTCA
AHC 16−6517 (J34)	2016-12-20	Sechelt, BC	Adult	18	Male	SRKW	Liver	3	NA	4.32	Trauma, possible vessel strike, left thorax.	175.3, 4057.2	PFOSA
AHC 18−6458	2018-11-14	Nootka Island, BC	Neonate	0.1[a]	Unknown	Bigg's	Liver	3	NA	3.96	Failure to thrive, possible fetal distress, hypoglycemia and emaciation.	591.7, 14942.5	7:3 FTCA

[a]All neonates were considered to be 1 month old (~0.1 year). Note: SRKW = southern resident killer whale, NA = not available.

Little is known about the fate and consequences of many CECs (hereafter referring to AP, triclosan, methyl triclosan, and selected PFAS compounds) and new POPs (hereafter referring to PFOS, PFOA, PFHxS, and HBCDD) in killer whales. Some of these chemicals are persistent and have a strong propensity to be present in these mammals due to constant exposure or accumulate in various tissues. Their concentrations can biomagnify throughout the food web, threatening the health of higher trophic-level marine mammals.[11−14] Further background information on the contaminants targeted in this study can be found in the Supporting Information.

In the northeastern Pacific (NEP) ocean, there are three recognized killer whale (*Orcinus orca*) ecotypes: Bigg's (Transient), Resident, and Offshore killer whales, each being genetically distinct with different behaviors, culture, socialization, and feeding ecology.[15,16] Facing a population extinction probability of 26%,[14] the Southern Resident killer whale (SRKW) is considered one of the most endangered marine mammals in the world. With a small population of approximately 73 to 74 individuals, its main threats include scarce food availability (i.e., quantity and quality of its main prey, Chinook salmon), anthropogenic disturbances (i.e., maritime traffic, acoustic pollution), and chemical pollution.[17,18]

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Recently, CECs and new POPs have been detected in killer whales stranded in Greenland and Norway and other odontocetes in New Zealand, with maternal transfer and feeding ecology playing an important role in contamination burdens.[19−21] Recent research on legacy contaminant exposure in SRKW critical habitat and POP modulation in SRKWs showed effects on lipid-remobilization, reproductive hormones, pregnancy failure, and viable calf production based on primary prey (Chinook salmon,*Oncorhynchus tshawytscha*) availability, fecal contaminant loads, and reproductive status.[11,18,22,23] As free-ranging killer whales are long lived, top predators, and have large amounts of fat storage, they are at higher risk of pollution accumulation and adverse health effects including population decline; recent research claims these species are among the most contaminated cetaceans in the world.[24−29]

Screening of selected CECs and new POPs has yet to be undertaken in any of the three ecotypes of known killer whale populations frequenting the coast of British Columbia (BC), Canada.[30] While our scientific understanding of the state of marine pollution in the NEP is limited,[11] the ubiquity of CECs and other legacy chemical contaminants suggests exposure and possible biomagnification in marine mammals inhabiting BC coastal waters.[31−33] A major objective of the resident killer whale recovery strategy outlined by Fisheries and Oceans Canada is to "ensure that chemical and biological pollutants do not prevent the recovery of resident killer whale populations".[18] Therefore, the aim of the present study is as follows: (1) conduct the first assessment of selected CECs and new POPs in liver and skeletal muscle (SM) samples collected from SRKWs and Biggs killer whales stranded from 2006 to 2018 in BC and (2) investigate *in utero* transfer of these pollutants in a pregnant SRKW. These data will provide regulatory policy new information to support risk management and control of specific contaminant sources and enhance the conservation of killer whales both in the NEP and globally.

MATERIALS AND METHODS

Ethics Declaration. The study involved the post mortem examination of dead and stranded killer whales under permit from the Department of Fisheries and Oceans Canada (DFO licence number XMMS 2 2021) with no live animal capture or sampling.

Tissue Sampling and Additional Data Collection. SM (n = 4) and liver (n = 10) samples were collected from twelve stranded individuals along the coast of BC from 2006−2018 (Figure S1) according to standardized necropsy protocols.[34] Morphometrics were compiled and the stranding location, date, age category and estimates (six adults, one juvenile, four neonates, one fetus), sex (five males, six females, one unknown), ecotype (six SRKWs, six Bigg's killer whales), class, and carcass condition code were recorded, following protocols by Raverty et al.[35] (Table 1). Individual animals were identified, and age classes were assigned according to morphometrics, photo-identification, and comparison with individual distinguishing features detailed in population catalogues.[36] The three ecotypes of killer whales described in the NEP were determined based on morphology via long-term photo-identification, dietary preference, genetics, social organization/culture, behavioral traits, vocal habits, and geographic range.[15,16,37] Further sample collection protocols can be found in the Supporting Information.

A carcass condition code and body condition index (BCI) for killer whales were obtained through published data and case reports.[38] Carcass condition code criteria are based on Geraci & Loundsbury:[39] 1 is a live animal; 2 is freshly dead; 3 is fair condition (early stage of decomposition, but organs essentially intact); 4 is in poor condition (advanced decomposition); and 5 is a mummified carcass or skeletal remains. BCI was calculated as a function of the individual killer whale's girth and length (i.e., BCI = girth/length) and ranges from poor (BCI = 0.5−0.6) to good (BCI = 0.6−0.7) values.[38] Individuals that may have been artificially inflated to mimic bloating or pregnancy have a BCI ranging from 0.7−0.8. BCI data were obtained for five individuals (Table 1).

Analytical Methods. The signalment, tissue sample inventory, and ecotypes of archived killer whale samples were transported to SGS AXYS Analytical Services Ltd. (Sidney, BC) for organic chemical analysis. Tissue samples were processed for a total of 49 contaminants, including four AP [4-nonylphenol (4NP), 4-*n*-octylphenol (4nOP), nonylphenol mono-ethoxylate (NP1EO), and nonylphenol di-ethoxylate (NP2EO)], three HBCDD (alpha, beta, and gamma HBCDD), triclosan, methyl triclosan, and 40 PFAS (see Table S1 for a full list of analytes and raw data for each contaminant). The description of the analytical procedures for the target contaminants and quality assurance/quality control details can be found in the Supporting Information.

Data Treatment and Statistical Analysis. Data treatment and statistical analyses were performed using RStudio version 4.0.2. Contaminant concentrations were blank-corrected by subtracting the concentration in the associated method blank [method detection limit (MDL)] for each analyte from the sample concentration to account for background contamination throughout laboratory analyses (see Table S1 for MDL values). Samples with no contaminant detection were substituted with either 1/2 half of the blank concentration ([blank]/2) or blank reporting limit (RL) divided by the square-root of two ($RL/\sqrt{2}$) if no blank contaminant concentration was detected.[20,40,41] These adjusted samples and those analytes with contaminant concentrations below the associated blank were not blank corrected. Contaminants that were equal in concentration to the blank sample were replaced with values derived from the [blank]/2 calculation. Sample RLs for AP data ranged from 0.478 to 12 ng/g wet weight (ww); HBCDD from 0.0933 to 0.177 ng/g ww; triclosan from 0.0002 to 0.0005 ng/g ww; methyl triclosan from 0.0004 to 0.0025 ng/g ww; and PFAS from 0.093 to 3.36 ng/g ww (Table S1). If more than 50% of samples were reported as not detected (ND) for a given contaminant, the contaminant was no longer considered in the present study.[20,42] Taking this into account, only 21 out of the 49 total contaminants were analyzed in this study.

Contaminants are reported in wet weight (ng/g ww) with the exception of HBCDD, which is reported in lipid weight (ng/g lw) unless otherwise stated (Table S2). Wet weight reporting is the most rational and frequent type of reporting in the literature for most CECs and protein binding PFAS, while lipid weight is generally used in conjunction with wet weight when reporting concentrations for lipophilic and hydrophobic compounds. For example, PFAS contaminants are normally reported in wet weight as these substances preferentially associate with proteins, while HBCDD compounds primarily associate with lipids (lipophilic). Therefore, in the present study, HBCDD was reported on a lipid-weight basis.

All statistical data analyses used a significance level of 0.05 (α = 0.05). The contaminant data were tested for normal

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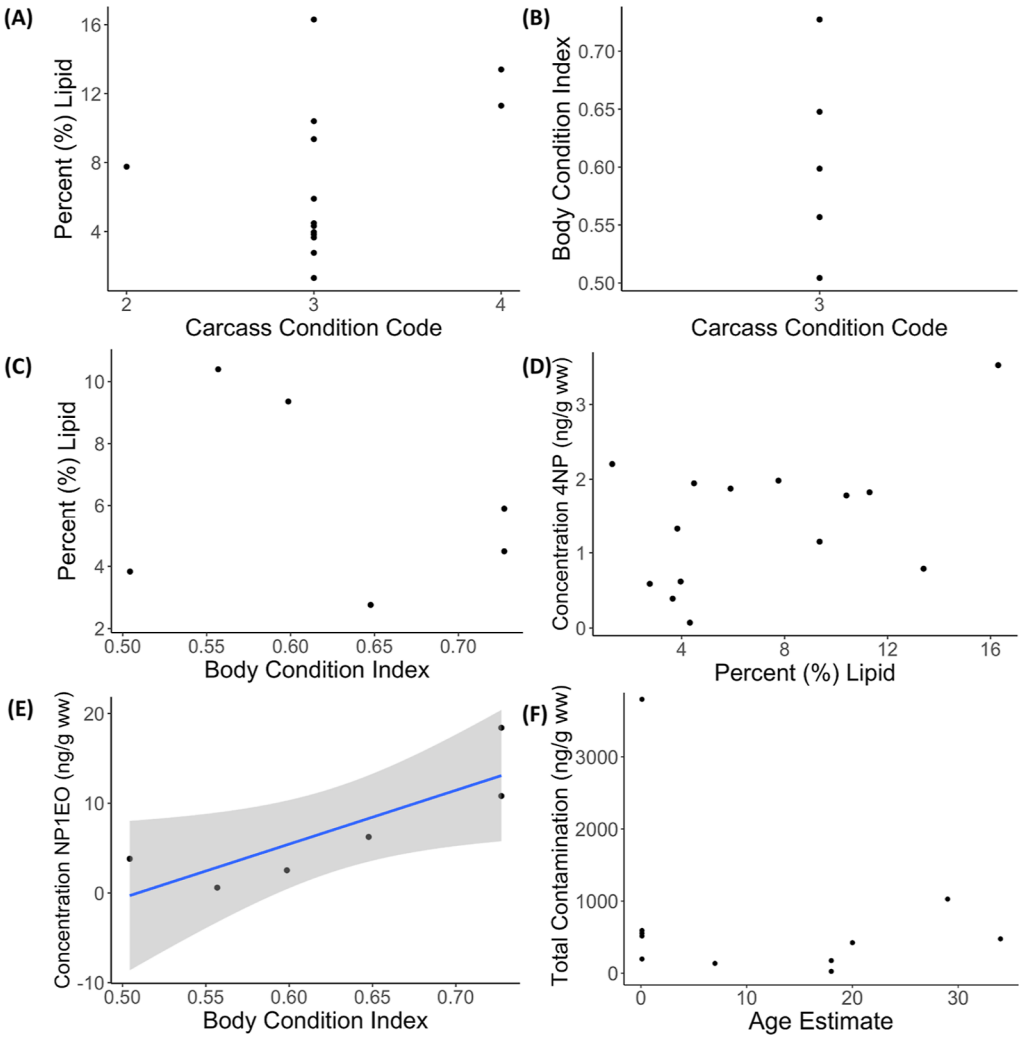

Figure 1. Confounding variable correlation analyses. (A) Relationship between carcass condition code and percent (%) lipid in each killer whale sample (p = 0.21). (B) Relationship between carcass condition code and body condition index (BCI). (C) Correlation of BCI with percent (%) lipid (p = 0.62). (D) Relationship between percent (%) lipid and contaminant concentration (4NP as an example, p = 0.26). (E) Significant positive relationship between BCI and contaminant concentration (NP1EO as an example, p = 0.041, r = 0.83). (F) Correlation between age estimate and total contamination concentration in each sample (p = 0.46). Note in (F): the age of two killer whales are unknown (Table 1) and were thus not included, and HBCDD is included as wet weight. Carcass condition code criteria are based on Geraci & Loundsbury:[39] 1 is a live animal; 2 is freshly dead; 3 is fair condition (decomposed but organs basically intact); 4 is in poor condition (advanced decomposition); and 5 is a mummified carcass or skeletal remains. BCI is a function of the individual killer whale's girth and length (BCI = girth/length) and ranges from poor values (BCI = 0.5−0.6) to good (BCI = 0.6−0.7).[38] Individuals that may have been artificially inflated to mimic bloating or pregnancy have a BCI ranging from 0.7−0.8. Best-fit lines are denoted in blue with 95% confidence levels shown in dark gray for significant correlations only. CEC and New POP Exposure in SRKW and Bigg's Killer Whales.

distribution using the Shapiro Wilk test, and for homogeneity of variance using the Brown−Forsythe test. Correlation analyses (Pearson or Spearman) were used to examine correlations between carcass condition codes, lipid content, BCI, and contaminant concentrations. For comparisons between variables, the non-parametric Wilcoxon Rank Sum Exact test was applied to non-normal data, while the parametric Welch's Two Sample t-test was used for normally distributed data.

Maternal Transfer Assessment. For assessing *in utero* transfer of CECs and new POPs, a mother-fetus SM sample pair, AHC 14−5855 (J32 Mother) and AHC 14−5856 (J32 Fetus) (Table 1), was available. Maternal transfer ratios (MTRs) were based on SM contaminant concentrations and calculated for each contaminant [i.e., (contaminant concentration in J32 Fetus SM)/(contaminant concentration in J32 Mother SM)] to assess the proportion of contaminant concentrations observed in the fetus relative to concentrations in the mother. Any resulting values above one (i.e., MTR > 1) indicate efficient and preferential exposure of the contaminant from the mother to the fetus through the placenta, while MTR < 1 for a given contaminant is indicative of scarce or lack of maternal transfer. Any contamination detected in J32 Fetus demonstrates maternal transfer. These ratios were then correlated with log K_{ow} values corresponding to the respective contaminant to explore whether K_{ow} (a criterion of contaminant lipophilicity and bioaccumulation potential) influenced transplacental maternal transfer of contaminants. Maternal transfer rates (%) were also calculated based on SM mass contaminant concentrations by applying the following formula reported in Gebbink et al.:[20] [contaminant concentration (ng/g) in J32 Fetus]/[contaminant concentration (ng/

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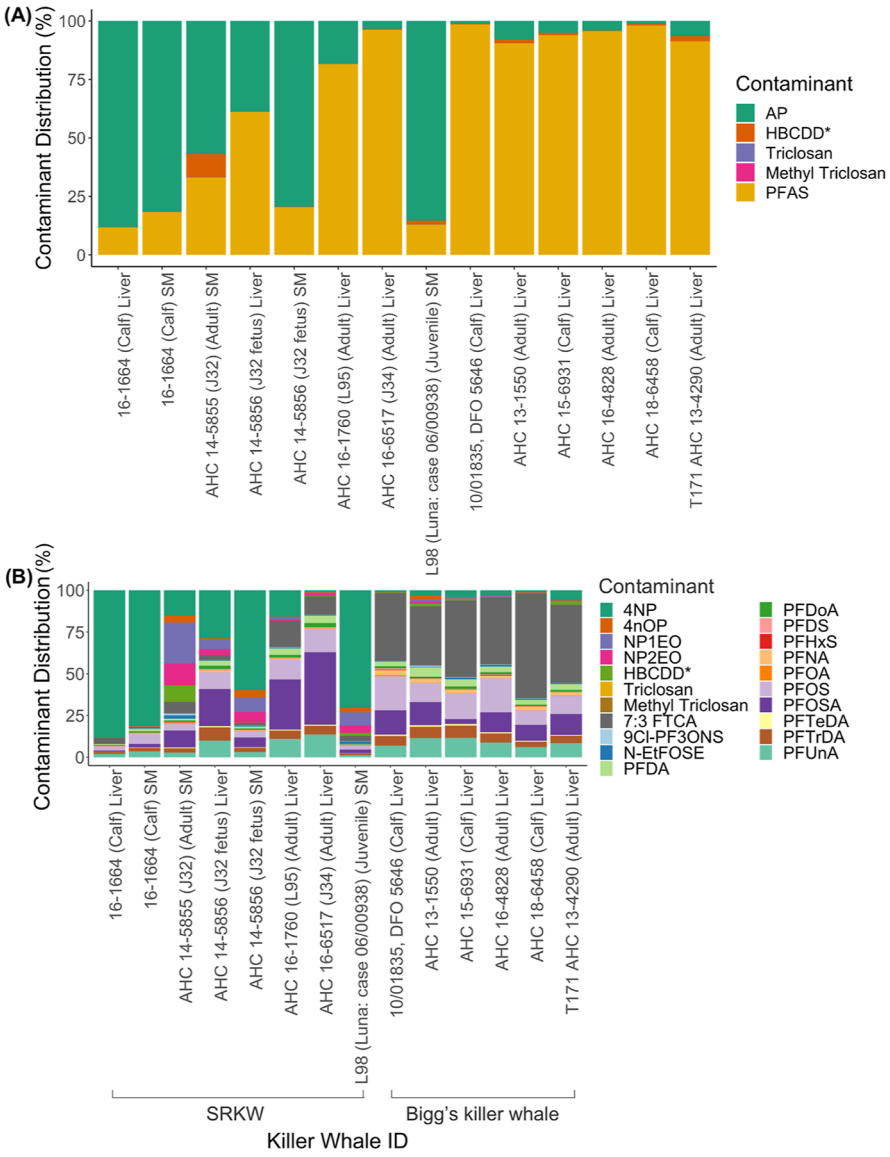

Figure 2. Patterns of contaminants (wet weight) in SRKW and Biggs' killer whale (*O. orca*) samples (n = 14, SM and liver) analyzed in this study. (A) Overall summary distribution (%) of CECs (i.e., AP, HBCDD*, methyl-triclosan, PFAS, and triclosan) analyzed in liver and SM tissue samples. (B) Detailed analyte-specific composition and distribution (%) analyzed in liver and SM tissue samples. *HBCDD is included as wet weight.

g) in J32 Mother + J32 Fetus] x 100. Note that contaminant burdens could not be calculated as total SM mass measurements were not available.

RESULTS AND DISCUSSION

Controlling for Confounding Variables. Correlations between lipid, BCI, carcass condition codes, and age were evaluated to determine if these variables influenced contaminant concentrations. Carcass condition codes and lipid content across all samples were not significantly correlated (Figure 1A), indicating that carcass condition did not influence lipid content. Similarly, carcass condition codes and BCI were not significantly correlated (Figure 1B), suggesting that the stage of carcass decomposition did not affect BCI. No significant correlation was found between BCI and lipid content (Figure 1C), indicating that BCI did not affect lipid content in the killer whale samples despite a positive trend (higher lipid content and BCI values) being indicative of relatively healthier animals. Nonetheless, some individuals may have been inflated by bloating due to carcass decomposition which may have artificially increased BCI values (>0.7 or 0.8). Contaminant concentrations showed no significant correlations with sample lipid content (4NP presented in Figure 1D as an example).

BCI showed a significant correlation with four contaminants (Figure S2): NP1EO (p = 0.041, r = 0.83), NP2EO (p = 0.032, r = 0.89), 9Cl-PF3ONS (p = 0.049, r = −0.83), and *N*-EtFOSE (p = 0.036, r = −0.84), suggesting a plausible association between BCI and contaminant concentration observed in these samples (NP1EO presented in Figure 1E as an example). In the case of NP1EO and NP2EO, a good BCI value (BCI = 0.6−0.7) was related to a higher contaminant concentration. Conversely, 9Cl-PF3ONS and *N*-EtFOSE showed a negative correlation with BCI.

Discrepancies found between relationships of carcass condition codes and BCI with contaminant loads may be due to carcass condition values and/or states of decomposition. For example, PFAS contamination has been shown to

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be dependent on the contaminant type, the organism in which it is found (i.e., size), and the recovery time of the carcass (i.e., duration of decomposition).[43−45] Interestingly, some PFAS substances such as PFOS can be formed in the process of degradation. Therefore, such extraneous variables may be impacting the contaminant concentrations in these killer whales.

The present study did not show significant differences in contaminant concentrations between males (n = 5) and females (n = 8) nor between age and total contamination in these killer whale samples (Figure 1F). Nonetheless, it was interesting to observe the trends of these correlations. Four neonates (one SRKW and three Bigg's killer whales with an estimated age of ∼1 month or ∼0.1 year) exhibited higher total contaminant concentrations compared to the concentrations measured in a juvenile (7 years) and adult animals (ages ranging from 18 to 34 years; Table 1) with the exception of T171 AHC 13−4290, a 29 year old female. Neonate contaminant concentrations exceeded those observed in the eldest individual (Bigg's killer whale; Table 1). High contaminant concentration levels observed in neonates relative to the juvenile and adult individuals (Figure 1F) may be due to the onset of sexual maturity and reproduction in subadult/adult individuals of these killer whale ecotype populations,[26,46,47] as well as the influence of contaminant maternal transfer processes (e.g., *in utero* transfer to fetus, neonate lactation), which is further discussed hereafter. Overall, few significant correlations were identified between these confounding variables in relation to contaminant concentration and did not impact contaminant prevalence in the studied samples.

The relationships discussed here may be influenced by sex, age, ecotype, tissue sample type, and underlying pathologies and health status of the animals.[21,38,46,47] Nutritional stress may also influence these relationships; SRKWs face scarce availability of their main prey, Chinook salmon, which may ultimately modulate lipid reserves and contaminant burdens through processes such as lipid mobilization.[18,22,31,48,49] Along with a small sample size, these aspects may reduce statistical power of these results.

Alkylphenol. Four alkylphenol contaminants were screened in each SM and liver sample. Both 4NP and NP2EO were detected above RL (>RL) in all samples, whereas 4nOP and NP1EO were not detected at RL in two samples (Table S1). 4NP sample concentrations ranged from 1.8 to 3344.94 ng/g ww (median 40.84 ng/g ww); 4nOP from 0.035 to 5.69 ng/g ww (median 1.21 ng/g ww); NP1EO from 0.07 to 18.41 ng/g ww (median 2.66 ng/g ww); and NP2EO from 0.21 to 10.9 ng/g ww (median 2.058 ng/g ww) across all samples (Table S2). The sum of the total AP concentration in each sample accounted for 47.76% of the total contaminant concentrations (Figure 2A), with 4NP accounting for 96.67% of the total AP concentration (Figure 2B).

Past studies have focused on testing AP presence in bivalves, gastropods, and fish, and there is a paucity of AP screening and detection in marine mammals.[50−53] Klosterhaus et al.[50] reported concentrations of APs detected in mussels found in San Francisco (California, USA) that were 1−2 orders of magnitude lower than the concentration expected to elicit toxic effects in marine organisms. Although risk management strategies for these compounds were established in 1999, results from David et al.[51] indicated that even after institution of contamination regulation, biomagnification of APs continues to accrue in higher trophic levels. An enhanced understanding of how these compounds enter into and are distributed throughout critical killer whale habitat may better inform or refine mitigation strategies.

Compared to other AP contaminants screened in this study, 4NP had the highest concentration and was the most prevalent contaminant in several tissue samples [i.e., 16−1664 liver and SM, J32 Fetus SM, and L98 (Luna: case 06/00938) SM; Figure 2B]. Alkylphenol ethoxylates (APEOs) are primarily incorporated into herbicides, pesticides, lubricating oils, and surfactants, and can biodegrade to nonylphenols (NPs), including 4NP.[7,51] Similar to other compounds in this contaminant class that are released to the environment though sewage treatment plants and industrial runoffs, sources of 4NP in seawater are derived primarily from the degradation of commercial and industrial products and sewage. High concentrations of this contaminant have been reported in toilet paper, especially those products high in recycled-paper content.[54,55] According to assessments by Diehl et al.,[54] Morro Bay (California, USA) had the highest 4NP levels measured in septic sludge (3750 mg/kg dry weight), followed by Canada at 4.6−1230 mg/kg dry weight. Killer whale contaminant concentrations in this study (751.78 ± 422.32 ng/g lw) were lower compared to those in organisms of Morro Bay, where levels ranged from 14000 ± 5600 ng/g lw in harbor porpoise (*Phocoena phocoena*) liver and 138000 ± 55000 ng/g lw in sea otter (*Enhydra lutris*) liver samples.[54]

Due to reproductive, developmental, and endocrine health implication of AP contaminants, as well as 4NP's specific ability to interact with the nervous system and influence cognitive function,[51,56] it imperative to better define the prevalence and potential impacts of these compounds in marine mammals.[57,58] Under the European Chemical Agency (ECHA), 4NP manufacture in or imported to the European Union (EU) is restricted based on weight, and NPs and their ethoxylates have also been added under the List of Toxic Substances by the Canadian Environmental Protection Act (CEPA). These compounds are also regulated in many Asian countries such as Singapore and China. Although regulation proposals are under consideration, there are currently no specific restriction in place for NPs under the Unites States Environmental Protection Agency (EPA).

Hexabromocyclododecane. All tissue samples were screened for alpha-, beta-, and gamma-HBCDD. Beta- and gamma-isomers were not detected at RL in any sample (Table S1). Alpha-HBCDD was not detected at RL in three samples (J32 Fetus liver, AHC 16−1760 (L95) liver, and 16−1664 SM). Lipid normalized concentrations of HBCDD ranged from 0.63 to 226.92 ng/g lw (median 23.89 ng/g lw; Figure S3) and accounted for 0.61% of the total contaminant lipid weight concentrations across all samples (Table S2). For reference of HBCDD wet weight distribution in each sample, see Figure 2A,B.

HBCDD concentrations found here were consistent with HBCDD levels in prior marine mammal research.[59] In a recent study, analyzing this contaminant in killer whales off the coast of Norway, concentrations ranged from not detected (ND) to 196 ng/g lw in blubber and 50 to 360 ng/g lw in muscle samples.[19] Likewise, Lam et al.[59] reported HBCDD concentrations ranging from 32 to 519 ng/g lw and 4.1 to 501 ng/g lw for Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*), respectively. Cetacean species from the Northern Pacific Ocean also

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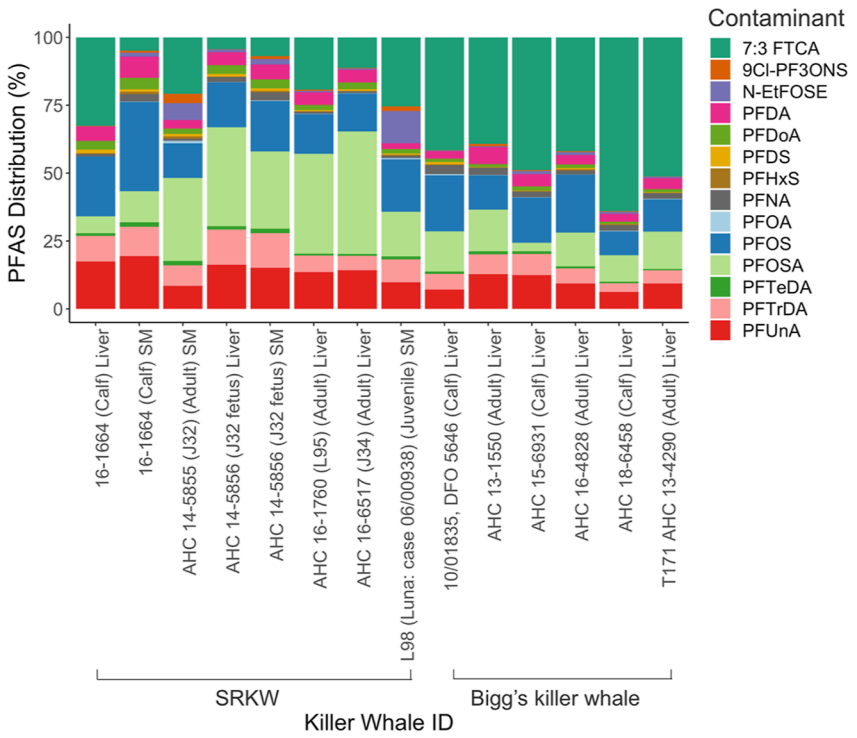

Figure 3. Per- and polyfluoroalkyl substances (PFAS) contaminant concentration (wet weight) distribution summary (%) for each killer whale (*O. orca*; SRKW and Bigg's killer whale) sample (n = 14, SM and liver). The composition pattern shows that 7:3 FTCA accounted for a high proportion of total PFAS concentrations, mainly in killer whale liver samples.

presented HBCDD concentrations similar to those found in the present study; for instance, HBCDD concentrations in bacon samples of Baird's beaked whale (*Berardius bairdii*) and bottlenose dolphin (*Tursiops truncatus*) from Japan ranged from 25 to 256 ng/g lw, respectively.[60] Concentrations of this contaminant were also reported in harbor porpoises and the common dolphin (*Delphinus delphis*) stranded in Europeans seas,[8,61] where the coasts of Ireland and Scotland presented high values (median of 2900 ng/g lw, maximum 9600 ng/g lw) with lower concentrations detected along the south coast of Ireland (median 1200 ng/g lw), the Netherlands (median 1100 ng/g lw), and both Belgium and the North Sea (770 ng/g lw). These concentrations were higher than those detected in the present study.

Differences in HBCDD levels in various geographic regions have been attributed to regional HBCDD application and use. For example, in the early 2000s, there was a greater demand and, therefore, use of these compounds in Europe than in America.[62] Although regulated under the Stockholm convention, this contaminant can still be found as flame retardant additives in clothing, building insulation, furniture textiles, and electrical equipment, and can easily be released to the environment through leaching and weathering.[59,63] Only some governing bodies have taken measures to further restrict, manufacture, use, sale, and import HBCDD (e.g., CEPA and ECHA have banned the manufacture and import of this contaminant). Results from this study indicate alpha-HBCDD is ubiquitous and bioaccumulative in apex marine mammals, such as the endangered SRKW and Bigg's killer whales.

Triclosan and Methyl Triclosan. Triclosan was detected in all SRKW and Bigg's killer whale samples, whereas methyl triclosan was identified in all but three samples (Table S1). Triclosan concentrations ranged from 0.003 to 0.43 ng/g ww (median 0.053 ng/g ww) and methyl triclosan ranged from 0.0005 (MDL 0.0006) to 0.085 ng/g ww (median 0.004 ng/g ww; Table S2). Triclosan accounted for 0.016% of the total contaminant concentration across all samples, while methyl triclosan accounted for 0.0017% (Figure 2A,B).

These results were consistent with prior studies analyzing triclosan in cetaceans. Plasma samples from free-ranging bottlenose dolphins (*T. truncatus*) off the coast of South Carolina and Texas, USA, showed a detectable presence of triclosan, with mean concentrations ranging from 0.18 to 0.072 ng/g ww depending on the geographic location.[64] This contaminant has also been measured in blood samples of a captive Bigg's killer whale, in which triclosan was ranked to be the third highest contaminant concentration (9.0 ng/g ww), following polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) compounds.[65] In this whale, triclosan accumulation was attributed primarily to its herring-based diet harvested from the NEP. This observation suggests that even with a disproportionately small percentage of herring in the diet of free ranging killer whales, this prey species may be a contributor to triclosan exposure and accumulation in these mammals.

Triclosan, a pharmaceutical and personal care product (PPCP), is prevalent in society as an antibacterial agent that can be found in consumer products such as toothpaste, soaps, detergents, toys, and cleaning products, and may enter the marine environment through residential wastewater and sewage effluent.[66,67] Regulatory actions of triclosan have been taken by CEPA, ECHA, and the US Food and Drug Administration. Certain countries including Japan have maximum allowable limits of triclosan in consumer products.[68] To the best of our knowledge, this is the first study to report methyl triclosan in free-ranging cetaceans along the BC coast and the first to report the presence of triclosan in SRKWs and Bigg's killer whales.

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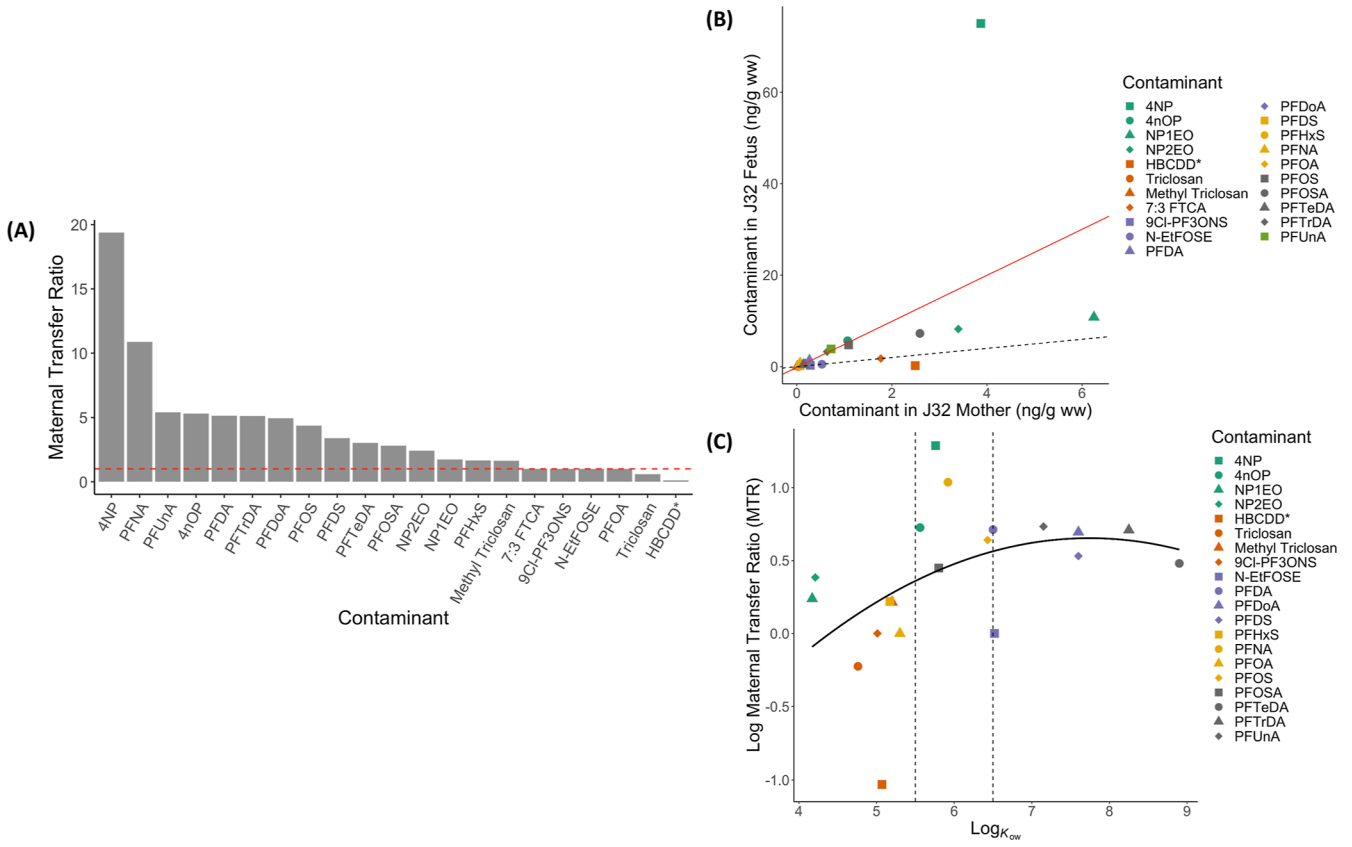

Figure 4. Analysis of contaminants (wet weight) in SM samples of the SRKW mother-fetus pair (J32 Mother) and J32 Fetus). (A) MTR calculated based on SRKW (*O. orca*) J32 Mother and her calf, J32 Fetus, contaminant concentrations. Contaminants with MTR >1 indicate the given contaminant was efficiently and preferentially exposed to the fetus, while contaminants with MTR <1 suggest scarce of lack of maternal transfer. The dashed red line indicates MTR = 1, representing equal partitioning of contaminant concentrations between fetus and mother. (B) Relationship (red line; $p = 1.55 \times 10^{-6}$, $r = 0.83$, slope = 5.03) between respective contaminant concentrations in J32 Mother and J32 Fetus. The dashed line indicates a 1:1 concentration ratio between these individual samples. Contaminants above the 1:1 line indicate a higher contaminant concentration in J32 Fetus compared to J32 Mother. (C) Relationship between the octanol−water partition coefficient (log K_{ow}) and MTR of each contaminant derived from J32 Mother and J32 Fetus samples. Shown is the best-fit quadratic curve and the dashed lines highlight the log K_{ow} range in which higher MTR values are found. Note for (C): The data points are presented in log scale and a log K_{ow} value was not found for 7:3 FTCA. *HBCDD is presented in wet weight.

Per- and Polyfluoroalkyl Substances. Analysis of killer whale samples identified 14 of 40 PFAS contaminants as 26 PFAS congeners were below RL (<RL) in more than 50% of the samples. The sum of PFAS concentrations in each sample ranged from 8.48 to 938.69 ng/g ww (median 266.35 ng/g ww). Total contaminant concentration across all tissue samples comprised 51.63% PFAS (Figure 2A).

Interestingly, the PFAS accounting for the majority of the overall concentrations was 7:3-fluorotelomer carboxylic acid (7:3 FTCA), contributing to 41.32% of total PFAS contamination across all samples, followed by PFOS (15.86%) and PFOSA (15.43%), as shown in Figures 2B and 3. In contrast to prior studies of PFAS in marine mammals, PFOS was not the dominant PFAS in our samples (Figure 3; Table S2).[41,69−74]

Prior studies have quantified the presence of PFAS in a variety of marine mammal species. Mean PFAS concentrations of each sample in our study (315.05 ± 133.39 ng/g ww) were higher than those recorded in killer whales off the coast of Greenland (269 ± 90 ng/g ww).[20] For example, mean PFOS concentrations measured here (49.97 ± 11.29 ww) were lower than those observed in Greenland killer whales (122 ± 42 ng/g ww).[20] PFAS concentrations have been widely studied in toothed cetaceans, with mean concentrations ranging from 927 ng/g ww in plasma of bottlenose dolphins (*T. truncatus*) from Sarasota, Florida (US) to 1738 ng/g ww in bottlenose dolphin plasma of the Gulf of Mexico and the Atlantic Ocean.[41,70] Stockin et al.[21] reported a maximum of 6975 ng/g ww in coastal-estuarine Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) liver samples of Australia based on preliminary data reported in the gray literature by Stephens et al.[75] The levels in our killer whale samples were lower to those averages previously stated. As PFAS has previously been reported to be underestimated in marine mammals from the Northern Hemisphere, our results further contribute to the prevalence of this contaminant in threatened killer whales in the NEP.[72]

Spaan et al.[72] reported 7:3 FTCA in cetaceans for the first time, with the highest reported concentration found in killer whales from East Greenland (614 ± 49 ng/g ww). This is a considerably higher burden than 7:3 FTCA concentrations measured in other vertebrate species such as birds, fish, and human blood, but not as high as concentrations found in polar bears (~1000 ng/g ww).[72] In the present study, 7:3 FTCA concentrations ranged from 1.77 to 481 ng/g ww (mean 130.18 ± 41.07 ng/g ww). Bigg's killer whale T171 (AHC 13−4290 liver sample) exhibited the highest concentration and was

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comparable to those found in killer whales from East Greenland. In contrast to our findings, however, Spaan et al.[72] found higher PFOS concentrations compared to 7:3 FTCA.

PFAS contaminants, also referred to as 'forever chemicals' due to their strong and very long persistence in the environment, are widely used in industrial settings; they have hydrophilic and lipophilic properties which allows for their frequent application in food packaging materials, stain and water-repellent fabrics, cooking ware, and fire extinguishers.[5] The international mandate of the Stockholm Convention on POPs prompted many countries, such as Canada, the US, and European Nations, to recognize the need for PFAS regulation, specifically PFOS, PFOA, and PFHxS. There are no known restrictions for 7:3 FTCA; however, one of its potential precursors, 8:2 fluorotelomer alcohol (8:2 FTOH), is classified as a "PFAS of Interest" under the EPA and is part of a list of chemicals proposed as new POPs to the Stockholm Convention by ECHA. Other potential precursors to 7:3 FTCA include 8:2 fluorotelomer sulfonates, C8-based perfluoroalkyl phosphonic acids (PFPAs), polyfluoroalkyl phosphates (PAPs), and polyfluoroalkyl phosphate diesters (di-PAPs). The present study provides further evidence of the prevalence of PFAS contaminants in top marine predators, specifically killer whales found in the NEP.

Maternal Transfer. Our results indicated *in utero* maternal transfer of pollutants during fetal development of offspring. To assess the degree of contaminant maternofetal transfer, SM tissue samples from a mother-fetus pair (J32 Mother and J32 Fetus) were analyzed. Tissue analysis disclosed *in utero* maternal transfer of all contaminants, with efficient and preferential (long-term exposure; MTR >1) transfer of 15 contaminants from the dam to the developing fetus (Figure 4A). The top three highest ratios were 4NP (MTR = 19.39), PFNA (MTR = 10.89), and PFUnA (MTR = 5.4; Table S3). The highest *in utero* MTR was 4NP, almost two times higher than PFNA, with 4NP concentration reported at 75.04 ng/g ww in J32 Fetus and 3.87 ng/g ww in J32 Mother. HBCDD (ww and lw basis) and triclosan had MTR values below one, indicating scarcity or lack of maternal transfer of these chemical contaminants via placenta from J32 Mother to J32 Fetus. Maternal transfer rates of 4NP were the highest at 95.1% followed by PFNA (91.59%) and PFUnA (84.38%). These results suggests that maternal transfer is an exposure pathway of alkylphenols and PFAS contaminants to killer whale fetuses and can result in greater fetal assimilation compared to levels in the mother (Figures 4A,B).

To better understand the transfer of contaminants from J32 Mother to J32 Fetus, the relationship between MTRs and octanol−water partition ratios was explored. An octanol−water partition coefficient (K_{ow}) is a common bioaccumulation metric expressing the lipophilicity of a chemical contaminant. Because the contaminant must travel through hydrophilic and hydrophobic environments, a compound's lipophilic nature can affect absorption and distribution throughout an organism.[76] A compound's lipophilic nature and trophic magnification factor tends to increase with K_{ow}.[13] Results from this study showed a significant positive correlation between log K_{ow} and MTR (p = 0.015, r = 0.54) and suggest that contaminants with a higher log K_{ow} (log K_{ow} > 5.5) were more readily transferred across placental membranes compared to contaminants with a lower log K_{ow} (i.e., log K_{ow} < 5.5, as shown in Figure 4C). In particular, contaminants such as 4NP and PFNA with 5.5 < log K_{ow} < 6.5 showed the highest MTRs (Figure 4C and Table S3).

Conversely, relatively lower MTR values are also observed with log K_{ow} ≥ 6.5. As a comparison, for instance, lower transplacental transfer ratios for PCBs were reported above a log K_{ow} of 7.5 in Steller sea lions (*Eumetopias jubatus*), beluga whales (*Delphinapterus leucas*), and California sea lions (*Zalophus californianus*).[77−79] The degree of chlorination or fluorination (i.e., lower or less chlorinated or fluorinated vs more persistent chlorinated or fluorinated compounds) in tandem with the contaminants' molecular weight (i.e., low molecular weight vs high molecular weight) are factors that may influence the transplacental transfer across *in utero* membranes. PFAS contaminants are known to partition with protein-rich compartments (e.g., blood and liver), with longer fluorinated compounds having higher bioaccumulation potential; it has been demonstrated that such PFAS contaminants are readily transported to human follicle fluid.[80,81] This is supported in the present study as long fluorinated carbon-chained PFAS contaminants such as PFDA, PFUnA, and PFDoA showed higher MTRs from J32 Mother to J32 Fetus (Figure 4C). Additionally, difference in molecular weight between 4NP and PFTeDA (493.76 kg/mol) may have contributed to 4NP being over six times more transferable than PFTeDA (Figure 4A,C).

This study is the first to document *in utero* maternal transfer of selected CECs and new POPs in killer whales inhabiting the NEP. Maternal transfer of PFAS has been studied in marine mammals in which contaminant loads in fetuses and neonates have been compared to dams in cetaceans and pinnipeds.[70,82−85] Few killer whale MTR analyses have been completed; however, Andvik et al.[19] reported maternal transfer of PFAS in a mother-fetus killer whale pair from Norway and claimed lipid rich milk and transplacental transfer were responsible for contaminant exposure. A study on a mother-fetus killer whale pair from Greenland also documents maternal transfer; however, contrary to the present study, all contaminant burdens were higher in the mother compared to the fetus.[20] Although there is limited ability to compare results with Gebbink et al.[20] as contaminant burdens were not calculated in the present study (see Methods), aspects impacting differences in MTR results between studies are discussed below.

Maternofetal transfer of contaminants is important to understand as calves are sensitive to toxicity in development and are at increased risk of pollutant exposure before birth. Additional mother-fetus pairs are needed to further assess maternal transfer of contaminants in killer whale species as discrepancies were found between the results of the present study and other studies analyzing maternal transfer in humans and cetaceans. For example in humans, Midasch et al.[86] reported consistently higher concentrations of PFOS in maternal-sampled blood compared to umbilical cord plasma samples, while PFOA showed only minor differences in concentration between the two sample types. This indicates the ability of PFOA to cross the placental barrier unhindered and contradicts the findings of our study as PFOS was lower in J32 Mother compared to J32 Fetus and PFOA had equal concentrations in the mother-fetus pair. Conversely, similar to that in the present study, Grønnestad et al.[85] found that sulfonated PFAS contaminants (e.g., PFOS) are more readily transferred across placental barriers compared to carboxylated PFAS contaminants (e.g., PFOA) in hooded seals (*Cystophora*

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cristata), and differences were attributed to protein-binding efficiencies and compound-specific persistence and retention. Also in agreement with the present study were higher concentration of PFHxS, PFOS, PFDoA, and PFTrDA found in hood seal pups compared to mothers which implies these compounds may be more readily eliminated from the mother (e.g., through metabolism, excretion, and placental transfer), while the fetus is unable to biotransform and eliminate such contaminants. This presents differences in detoxification mechanisms in J32 Mother and J32 fetus as a potential source of MTR variation in this pair.

Research regarding *in utero* transfer of 4NP has been performed in human samples, supporting the transfer of this contaminant from the mother to the fetus.[87−89] Li et al.[89] detected approximately 20% lower non-POP (e.g., 4NP) contaminant concentrations in the umbilical cord than maternal blood samples compared to polybrominated diphenyl ether (PBDE) compounds which showed significantly higher concentrations in umbilical-cord blood. It was concluded that the placental barrier provided only a slight decrease in non-POP contaminant exposure to fetuses. Although PBDE compounds were not studied here, 4NP was much higher than all other contaminants analyzed (Figure 4A) which suggests little protection from 4NP by the placental barrier. Other factors such as foraging in contaminant hotspots in critical habitats may have contributed to the high 4NP levels found in J32 Fetus.[84] General aspects potentially impacting differences in MTR between individuals and species include placental thickness, diffuse placentation, blood pH, and lipid solubility.[90]

The high MTR of 4NP in the present study may be considered an outlier in the data; however, given few studies have analyzed the presence of 4NP in cetaceans and MTR findings here are based on a sample size of one, it is difficult to confidently make such conclusions. Inconsistencies in results between studies and sparce research regarding *in utero* maternal transfer of CEC (such as 4NP) and new POP contaminants in different ecotype of killer whales with diverse feeding and dietary preferences (e.g., fish-eating vs marine mammal-eating) further emphasizes the need for additional studies on this topic to make more robust interpretations of the results found in this study.

Killer Whale Calf Population Contaminant Comparisons and Killer Whale Exposure Sources. Concentrations of CECs and new POPs in calf samples of SRKW (n = 3) and Bigg's killer whales (n = 4) were compared to explore for differences in contaminant concentrations. The calf cohort of SRKWs and Bigg's killer whales were grouped to include neonates and fetuses. The concentration of 4NP was significantly higher (p = 0.02) in SRKW calves, whereas HBCDD (p = 0.026, lipid weight basis), 7:3 FTCA (p = 0.04), PFHxS (p = 0.025), PFNA (p = 0.019), and PFOA (p = 0.044) concentrations were significantly higher in Bigg's killer whale calves (Figure S4). These findings may well indicate that SRKW calves were most exposed to a putative 4NP source nearby to their critical habitat relative to the more mobile Bigg's killer whale calves, which were most exposed to HBCDD and PFAS contaminants.

Significant differences in contaminant concentrations between SRKWs and Bigg's killer whale calves may be due to habitat.[91] Bigg's killer whales range throughout the west coast of North America, from Southeast Alaska to California, and transit both the outer coast and protected inshore areas, while SRKWs remain seasonally inshore or nearby coastal waters, with the Georgia and Johnstone Straight, BC, considered as their critical habitats.[16,18] A significantly higher concentration of 4NP in SRKWs may well be attributed to their primary habitats surrounding industrial and residential hubs as well as their association to a more estuarine trophic chain. As toilet paper is a major source of 4NP, sewage effluent may have exposed this species to elevated concentrations of this contaminant. Estuaries are considered heavily impacted by anthropogenic 4NP pollution; therefore, this CEC may have been more readily absorbed in SRKWs throughout the associated food web.[54] In contrast to SRKWs, Bigg's killer whales may be more exposed to HBCDD and PFOS in more remote regions as these contaminants have a strong propensity for long-range environmental and atmospheric transport and may be less exposed by local sources in the ocean.[4] These compounds are commonly found in flame retardant additives and PFOS specifically can be present in hydraulic fluids, electric parts, and textiles.

The unique presence of 7:3 FTCA in these killer whales warrants further investigation of potential sources and exposure pathways, including point and nonpoint pollution sources from urban, agricultural, or industrial areas at the regional level, as well as presumptive wet deposition via long-range atmospheric transport. 7:3 FTCA is a stable metabolite but can also be an intermediate product; for example, it can result from the metabolism of 8:2 FTOH.[92,93] Fluorinated telomer alcohols (FTOHs) are used as surfactants and in the production of PFAS contaminants and are prone to undergo atmospheric oxidation which can produce fluorotelomer carboxylic acids (FTCAs).[94] It is unclear whether FTOHs may serve as the precursor of 7:3 FTCA in the ocean atmosphere and marine environment of SRKW and Bigg's killer whales.

Contaminants such as legacy and emerging POPs and some CECs have the capacity to bioaccumulate in killer whales via biomagnification at each trophic level across their food webs;[31,33,91,95] thus, differences in contaminant concentrations in SRKW and Bigg's killer whale calf populations may also be attributed in part to foraging behavior and dietary preferences.[22,31,46,48,91,96] Fish-eating SRKWs had lower contaminant concentrations compared to marine mammal-eaters such as Bigg's killer whales in prior studies and the present study. This was exemplified in Norway where killer whales preying upon seals reported four times the concentration of PCBs than those feeding on fish.[24] In the NEP, SRKWs have shown significantly lower PCB and PBDE concentration levels compared to Bigg's killer whales, which has been attributed to the difference in trophic levels between these sympatric populations.[26,46,97] Chinook salmon, the primary prey of SRKWs, have shown PCB concentrations ranging from 516 to 3099 ng/g lw (estuary) and 521 to 760 ng/g lw (hatchery) while harbor seals, top constituents of Bigg's killer whale prey, had PCB levels ranging from 1,143 to 18,135 ng/g lw.[33,48,98,99] Based on extrapolation from pinniped studies, recent research has stated that PCB concentrations in these killer whale populations have surpassed the toxic effect concentration thresholds for PCBs (i.e., immunotoxicity and endocrine disruption).[24,46,91,95,100,101]

As both geographical location and food web composition and structure influence contamination in killer whales, this study highlights the need for further analyses of CECs and new

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POPs in the habitat and prey of these apex predators of the NEP.

Killer Whale Pathology and Association to CEC and New POP Exposure. Based on prior studies of harbor porpoises in the United Kingdom and bottlenose dolphins (*T. truncatus*) near Charleston, North Carolina (US), killer whales' chemical contamination may have surpassed thresholds and impacted homeostasis.[25,102] In free-ranging bottlenose dolphins, for instance, PFAS concentrations ranging from 500−9000 μg/L ww were associated with immunological and hematological alterations.[102] In our study, the top three highest concentrations of PFAS were detected in the liver of Bigg's killer whales, including two calves [i.e., 10/01835, DFO 5646 had 546.1 ng/g or part per billion (ppb) ww, and AHC 18−6458 had 580.8 ng/g ww] and one adult female (i.e., T171 AHC 13−4290 had ∼940 ng/g ww). This represents 21% of the total killer whales sampled, and exceeds the minimum range of 500 μg/L (ppb) associated with alterations on the haematology and circulating immune cell populations of bottlenose dolphins.

Additionally, in the bottlenose dolphins, elevated PFOS levels were associated with changes in phagocytic function and immune modulation,[102] which may have indirectly contributed to pathology, suboptimal health, and BCI in the sampled cohort of the present study (Table 1). Causes of killer whale mortalities in the present study ranged from infections, emaciation, blunt force trauma, dystocia, and intraspecific interactions.[38] In speculation, suboptimal body condition, coupled with elevated contaminant loads may have predisposed or exacerbated AHC 16−1760 (L95) to mucormycosis, a rare but serious fungal infection caused by *Rhizomucor pusillus*, *Lichtheimia corymbifera*, and *Cunninghamella bertholletiae*, recently observed in marine mammals stranded in the NEP.[103] In AHC 15−6931, it is difficult to infer a specific cause and effect between presumptive metabolic derangements, hypoglycemia, and dystocia[38,103] and elevated PFOSA and 7:3 FTCA, which were most dominant in this mammal (Table 1). At present, however, there is insufficient information regarding the impacts of CECs and new POPs to infer a specific cause and effect.

Many of these contaminants may have been associated or attributed to disruption of homeostasis. In humans, PFAS contaminants have been reported to alter immune and hepatic functions, disrupt glucose metabolism, and cause reproductive risks.[6,104] Studies have reported dystocia to be related to hormonal imbalances and endocrine disorders.[105,106] Moreover, alkylphenols have shown to alter the endocrine system which may create difficulties in the birthing process.[51]

As previously mentioned, the studied cohort is too small to assess variation in contaminant concentrations with antemortem, morbidity, reproductive failure, or the loss of these animals. Additionally, toxic thresholds for the analyzed CECs and most new POPs in marine mammals are not yet fluent in the literature. Further studies linking contaminant loads to pathological findings in a variety of marine mammal species are warranted.

Tissue analysis of necropsied SRKWs and Bigg's killer whales in the NEP demonstrated that CECs (AP, triclosan, methyl triclosan, and selected PFAS compounds) and new POPs (PFOS, PFOA, PFHxS, and HBCDD) are prevalent along the marine-coastal ecosystems of British Columbia, Canada. AP and PFAS pollutants were most common across killer whale samples. The AP group predominantly consisted of 4NP, a novel contaminant that has been studied in few marine mammal species; this is the first study to report 4NP levels in killer whales. Overall, 7:3 FTCA was the primary PFAS contaminant and was observed here for the first time in SRKWs and Bigg's killer whales. This contaminant was first detected in cetaceans in 2020;[72] therefore, little is known about the kinetics and metabolism of this contaminant in marine mammals. Interestingly, PFOS was not the dominant PFAS contaminant, as usually detected in marine mammals. Triclosan, methyl triclosan, and HBCDD accounted for a very small fraction of contamination across all samples. In addition to studying the prevalence of CECs in conjunction with new POPs regulated under the Stockholm Convention, we evaluated *in utero* maternal transfer of pollutants in the J32 Mother-J32 Fetus SM-sample pair. Efficient and preferential exposure of APs, particularly 4NP, and PFAS contaminants were detected in J32 Fetus (MTR >1). This raises concerns regarding the persistence of these emerging chemicals and potential impacts on fetal development and post-partum survival.

As previously discussed, it is difficult at this point to confidently assess both the prevalence and impacts of the studied contaminants in this species; the novel findings of this research should be considered as a preliminary baseline for future studies which can provide more robust and statistically convincing results through larger samples size analyses. Although access to skeletal muscle and liver samples of stranded and necropsied killer whales is uncommon and opportunistic, it is essential that contaminant distribution and prevalence continues to be monitored in endangered SRKWs and threatened Bigg's killer whales to expand our understanding of their ecotoxicological consequences. Additional toxicological risk assessments should also be done on these chemicals to support risk management and global regulation efforts of these substances. Nonetheless, the present study helps to establish baseline knowledge on CECs and new POPs in these charismatic and sentinel species, and our findings provide scientifically-derived evidence to inform policy to support and enhance regulations to mitigate pollutant exposure in marine ecosystems. These measures may also contribute to management strategies and conservation efforts of SRKWs and their critical habitat, as well as the habitat of other marine mammals living within BC's coastal ecosystem.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c04126.

Raw data for each sample (XLSX)

Wet and lipid-normalized weight data for each sample (XLSX)

Additional background information on the analyzed "contaminants of emerging concern" and new POPs; further procedure details regarding sample collection and contaminant analytical methods; killer whale stranding locations; panel of figures presenting significant correlations between BCI and contaminant concentration; lipid weight distribution of HBCDD for each sample; contaminant concentration comparisons in calves of different ecotypes; and MTRs for each contaminant and their associated octanol−water partition coefficient (log K_{ow}) (PDF)

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Notes

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REFERENCES

(1) Halpern, B. S.; Frazier, M.; Afflerbach, J.; Lowndes, J. S.; Micheli, F.; O'Hara, C.; Scarborough, C.; Selkoe, K. A. Recent Pace of Change in Human Impact on the World's Ocean. *Sci. Rep.* **2019**, *9*, 1−8.

(2) Landrigan, P. J.; Stegeman, J. J.; Fleming, L. E.; Allemand, D.; Anderson, D. M.; Backer, L. C.; Brucker-Davis, F.; Chevalier, N.; Corra, L.; Czerucka, D.; Bottein, M. Y. D.; Demeneix, B.; Depledge, M.; Deheyn, D. D.; Dorman, C. J.; Fénichel, P.; Fisher, S.; Gaill, F.; Galgani, F.; Gaze, W. H.; Giuliano, L.; Grandjean, P.; Hahn, M. E.; Hamdoun, A.; Hess, P.; Judson, B.; Laborde, A.; McGlade, J.; Mu, J.; Mustapha, A.; Neira, M.; Noble, R. T.; Pedrotti, M. L.; Reddy, C.; Rocklöv, J.; Scharler, U. M.; Shanmugam, H.; Taghian, G.; Van de Water, J. A. J. M.; Vezzulli, L.; Weihe, P.; Zeka, A.; Raps, H.; Rampal, P. Human Health and Ocean Pollution. *Ann. Glob. Heal.* **2020**, *86*, 151−64.

(3) Persson, L.; Carney Almroth, B. M.; Collins, C. D.; Cornell, S.; de Wit, C. A.; Diamond, M. L.; Fantke, P.; Hassellöv, M.; MacLeod, M.; Ryberg, M. W.; Søgaard Jørgensen, P.; Villarrubia-Gómez, P.; Wang, Z.; Hauschild, M. Z. Outside the Safe Operating Space of the Planetary Boundary for Novel Entities. *Environ. Sci. Technol.* **2022**, *56*, 1510−1521.

(4) Stockholm Convention on Persistent Organic Pollutants (POPs). *The 16 New POPs: An Introduction to the Chemicals Added to the Stockholm Convention as Persistent Organic Pollutants by the Conference of the Parties.* http://www.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx.

(5) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; de Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integr. Environ. Assess. Manag.* **2011**, *7*, 513−541.

(6) Sinclair, G. M.; Long, S. M.; Jones, O. A. H. What Are the Effects of PFAS Exposure at Environmentally Relevant Concentrations? *Chemosphere* **2020**, *258*, 127340.

(7) Shang, D. Y.; Macdonald, R. W.; Ikonomou, M. G. Persistence of Nonylphenol Ethoxylate Surfactants and Their Primary Degradation Products in Sediments from near a Municipal Outfall in the Strait of Georgia, British Columbia, Canada. *Environ. Sci. Technol.* **1999**, *33*, 1366−1372.

(8) Covaci, A.; Gerecke, A. C.; Law, R. J.; Voorspoels, S.; Kohler, M.; Heeb, N. V.; Leslie, H.; Allchin, C. R.; de Boer, J. Hexabromocyclododecanes (HBCDs) in the Environment and Humans: A Review. *Environ. Sci. Technol.* **2006**, *40*, 3679−3688.

(9) McKenzie, T.; Holloway, C.; Dulai, H.; Tucker, J. P.; Sugimoto, R.; Nakajima, T.; Harada, K.; Santos, I. R. Submarine Groundwater Discharge: A Previously Undocumented Source of Contaminants of Emerging Concern to the Coastal Ocean (Sydney, Australia). *Mar. Pollut. Bull.* **2020**, *160*, 111519.

(10) Krogh, J.; Lyons, S.; Lowe, C. J. Pharmaceuticals and Personal Care Products in Municipal Wastewater and the Marine Receiving Environment near Victoria Canada. *Front. Mar. Sci.* **2017**, *4*, 1−13.

(11) Alava, J. J. Legacy and Emerging Pollutants in Marine Mammals' Habitat from British Columbia, Canada: Management perspectives for sensitive marine ecosystems. In *Stewarding the Sound*; CRC Press, 2019; https://www.taylorfrancis.com/chapters/edit/10.1201/9780429025303-9/legacy-emerging-pollutants-marine-mammals-habitat-british-columbia-canada-management-perspectives-sensitive-marine-ecosystems-juan-jos%C3%A9-alava.

(12) Marine Mammal Ecotoxicology: *Impacts of Multiple Stressors on Population Health*, 1st ed.; Fossi, M. C., Panti, C., Eds.; Elsevier, Academic Press, 2018.

(13) Rayne, B. C.; Forest, M. G.; Blair, J. D.; Surridge, B.; Hoover, D.; Grace, R.; Gobas, F. A. P. C. Comment on "Perfluoroalkyl Contaminants in an Arctic Marine Food Web: Trophic Magnification and Wildlife Exposure". *Environ. Sci. Technol.* **2009**, *43*, 6112.

(14) Murray, C. C.; Hannah, L.; Doniol-valcroze, T.; Wright, B.; Stredulinsky, E.; Locke, A.; Lacy, R. *Cumulative Effects Assessment for Northern and Southern Resident Killer Whale (Orcinus Orca) Populations in the Northeast Pacific*, 2019.

(15) Ford, J. K. B.; Ellis, G. M.; Barrett-Lennard, L. G.; Morton, A. B.; Palm, R. S.; Balcomb, K. C. Dietary Specialization in Two Sympatric Populations of Killer Whales (*Orcinus orca*) in Coastal British Columbia and Adjacent Waters. *Can. J. Zool.* **1998**, *76*, 1456−1471.

(16) Ford, J. K. B.; Ellis, G. M.; Balcomb, K. C. *Killer Whales: The Natural History and Genealogy of Orcinus orca in British Columbia and Washington State*, 2nd ed.; UBC Press: Vancouver, British Columbia, 2000.
(17) Murray, C. C.; Hannah, L. C.; Doniol-Valcroze, T.; Wright, B. M.; Stredulinsky, E. H.; Nelson, J. C.; Locke, A.; Lacy, R. C. A Cumulative Effects Model for Population Trajectories of Resident Killer Whales in the Northeast Pacific. *Biol. Conserv.* **2021**, *257*, 109124.
(18) *Recovery Strategy for the Northern and Southern Resident Killer Whales (Orcinus orca) in Canada [Proposed]. Species at Risk Act Recovery Strategy Series*; Fisheries & Oceans Canada: Ottawa, 2018.
(19) Andvik, C.; Jourdain, E.; Lyche, J. L.; Karoliussen, R.; Borgå, K. High Levels of Legacy and Emerging Contaminants in Killer Whales (*Orcinus orca*) from Norway, 2015 to 2017. *Environ. Chem.* **2021**, *40*, 1848−1858.
(20) Gebbink, W. A.; Bossi, R.; Rigét, F. F.; Rosing-Asvid, A.; Sonne, C.; Dietz, R. Observation of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in Greenland Marine Mammals. *Chemosphere* **2016**, *144*, 2384−2391.
(21) Stockin, K. A.; Yi, S.; Northcott, G. L.; Betty, E. L.; Machovsky-Capuska, G. E.; Jones, B.; Perrott, M. R.; Law, R. J.; Rumsby, A.; Thelen, M. A.; Graham, L.; Palmer, E. I.; Tremblay, L. A. Per- and Polyfluoroalkyl Substances (PFAS), Trace Elements and Life History Parameters of Mass-Stranded Common Dolphins (Delphinus delphis) in New Zealand. *Mar. Pollut. Bull.* **2021**, *173*, 112896.
(22) Lundin, J. I.; Ylitalo, G. M.; Booth, R. K.; Anulacion, B.; Hempelmann, J. A.; Parsons, K. M.; Giles, D. A.; Seely, E. A.; Hanson, M. B.; Emmons, C. K.; Wasser, S. K. Modulation in Persistent Organic Pollutant Concentration and Profile by Prey Availability and Reproductive Status in Southern Resident Killer Whale Scat Samples. *Environ. Sci. Technol.* **2016**, *50*, 6506−6516.
(23) Wasser, S. K.; Lundin, J. I.; Ayres, K.; Seely, E.; Giles, D.; Balcomb, K.; Hempelmann, J.; Parsons, K.; Booth, R. Population Growth Is Limited by Nutritional Impacts on Pregnancy Success in Endangered Southern Resident Killer Whales (*Orcinus orca*).*PLoS One* **2017**, *12*(). DOI: 10.1371/journal.pone.0179824.
(24) Andvik, C.; Jourdain, E.; Ruus, A.; Lyche, J. L.; Karoliussen, R.; Borgå, K. Preying on Seals Pushes Killer Whales from Norway above Pollution Effects Thresholds. *Sci. Rep.* **2020**, *10*, 1−10.
(25) Jepson, P. D.; Deaville, R.; Barber, J. L.; Aguilar, À.; Borrell, A.; Murphy, S.; Barry, J.; Brownlow, A.; Barnett, J.; Berrow, S.; Cunningham, A. A.; Davison, N. J.; ten Doeschate, M.; Esteban, R.; Ferreira, M.; Foote, A. D.; Genov, T.; Giménez, J.; Loveridge, J.; Llavona, Á.; Martin, V.; Maxwell, D. L.; Papachlimitzou, A.; Penrose, R.; Perkins, M. W.; Smith, B.; de Stephanis, R.; Tregenza, N.; Verborgh, P.; Fernandez, A.; Law, R. J. PCB Pollution Continues to Impact Populations of Orcas and Other Dolphins in European Waters. *Sci. Rep.* **2016**, *6*, 1−17.
(26) Krahn, M. M.; Hanson, M. B.; Baird, R. W.; Boyer, R. H.; Burrows, D. G.; Emmons, C. K.; Ford, J. K. B.; Jones, L. L.; Noren, D. P.; Ross, P. S.; Schorr, G. S.; Collier, T. K. Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales. *Mar. Pollut. Bull.* **2007**, *54*, 1903−1911.
(27) Law, R. J.; Barry, J.; Barber, J. L.; Bersuder, P.; Deaville, R.; Reid, R. J.; Brownlow, A.; Penrose, R.; Barnett, J.; Loveridge, J.; Smith, B.; Jepson, P. D. Contaminants in Cetaceans from UK Waters: Status as Assessed within the Cetacean Strandings Investigation Programme from 1990 to 2008. *Mar. Pollut. Bull.* **2012**, *64*, 1485−1494.
(28) Remili, A.; Letcher, R. J.; Samarra, F. I. P.; Dietz, R.; Sonne, C.; Desforges, J. P. W.; Víkingsson, G.; Blair, D.; McKinney, M. A. Individual Prey Specialization Drives PCBs in Icelandic Killer Whales. *Environ. Sci. Technol.* **2021**, *55*, 4923−4931.
(29) Desforges, J. P.; Hall, A.; McConnell, B.; Rosing-Asvid, A.; Barber, J. L.; Brownlow, A.; De Guise, S.; Eulaers, I.; Jepson, P. D.; Letcher, R. J.; Levin, M.; Ross, P. S.; Samarra, F.; Víkingson, G.; Sonne, C.; Dietz, R. Predicting Global Killer Whale Population Collapse from PCB Pollution. *Science* **2018**, *361*, 1373−1376.
(30) Southern Resident Killer Whales. *Southern Resident Killer Whale Contaminants Technical Working Group Accomplishments Report*; Environment and Climate Change Canada (ECCC), 2020.
(31) Alava, J. J.; Cisneros-Montemayor, A. M.; Sumaila, U. R.; Cheung, W. W. L. Projected Amplification of Food Web Bioaccumulation of MeHg and PCBs under Climate Change in the Northeastern Pacific. *Sci. Rep.* **2018**, *8*, 1−12.
(32) Alava, J. J.; Gobas, F. A. P. C. Modeling 137Cs Bioaccumulation in the Salmon-Resident Killer Whale Food Web of the Northeastern Pacific Following the Fukushima Nuclear Accident. *Sci. Total Environ.* **2016**, *544*, 56−67.
(33) Cullon, D. L.; Yunker, M. B.; Christensen, J. R.; Macdonald, R. W.; Whiticar, M. J.; Dangerfield, N. J.; Ross, P. S. Biomagnification of Polychlorinated Biphenyls in a Harbor Seal (*Phoca vitulina*) Food Web from the Strait of Georgia, British Columbia, Canada. *Environ. Toxicol. Chem.* **2012**, *31*, 2445−2455.
(34) Raverty, S.; Gaydos, J.; Leger, J. *Killer Whale (Orcinus orca) Necropsy and Disease Testing Protocol*. https://www.seadocsociety.org/blog/killer=whale-necropsy=protocol-2014?rq=killer-whale-necropsy-protocol.
(35) Raverty, S.; Duignan, P.; Jepson, P.; Morell, M. Marine Mammal Gross Necropsy. *CRC Handb. Mar. Mammal Med.* **2018**, 249−268.
(36) Towers, J. R.; Sutton, G. J.; Shaw, T. J. H.; Malleson, M.; Matkin, D.; Gisborne, B.; Forde, J.; Ellifrit, D.; Ellis, G. M.; Ford, J. K. B.; Doniol-Valcroze, T.. *Photo-Identification Catalogue, Population Status, and Distribution of Bigg's Killer Whales Known from Coastal Waters of British Columbia, Canada*; Canadian Technical Report of Fisheries and Aquatic Sciences, 2019; Vol. *3311*.
(37) Barrett-lennard, L. G.; Ford, J. K. B.; Heise, K. A.; HEISE, K. A. The Mixed Blessing of Echolocation: Differences in Sonar Use by Fish-Eating and Mammal-Eating Killer Whales. *Anim. Behav.* **1996**, *51*, 553−565.
(38) Raverty, S.; St. Leger, J. S.; Noren, D. P.; Burek Huntington, K. B.; Rotstein, D. S.; Gulland, F. M. D.; Ford, J. K. B.; Hanson, M.; Lambourn, D. M.; Huggins, J.; Delaney, M. A.; Spaven, L.; Rowles, T.; Barre, L.; Cottrell, P.; Ellis, G.; Goldstein, T.; Terio, K.; Duffield, D.; Rice, J.; Gaydos, J. K. Pathology Findings and Correlation with Body Condition Index in Stranded Killer Whales (*Orcinus orca*) in the Northeastern Pacific and Hawaii from 2004 to 2013. *PLoS One* **2020**, *15*, No. e0242505.
(39) Geraci, J. R.; Loundsbury, V. J. *Marine Mammals Ashore: A Field Guide for Strandings*, 2nd ed.; Yates, N. S., Ed.; National Aquarium in Baltimore, 2005.
(40) Alava, J. J.; Calle, P.; Tirapé, A.; Biedenbach, G.; Cadena, O. A.; Maruya, K.; Lao, W.; Aguirre, W.; Jiménez, P. J.; Domínguez, G. A.; Bossart, G. D.; Fair, P. A. Persistent Organic Pollutants and Mercury in Genetically Identified Inner Estuary Bottlenose Dolphin (*Tursiops truncatus*) Residents of the Guayaquil Gulf, Ecuador: Ecotoxicological Science in Support of Pollutant Management and Cetacean Conservation. *Front. Mar. Sci.* **2020**, *7*, 122.
(41) Houde, M.; Wells, R. S.; Fair, P. A.; Bossart, G. D.; Hohn, A. A.; Rowles, T. K.; Sweeney, J. C.; Solomon, K. R.; Muir, D. C. G. Polyfluoroalkyl Compounds in Free-Ranging Bottlenose Dolphins (*Tursiops truncatus*) from the Gulf of Mexico and the Atlantic Ocean. *Environ. Sci. Technol.* **2005**, *39*, 6591−6598.
(42) Harris, K. A.; Nichol, L. M.; Ross, P. S. Hydrocarbon Concentrations and Patterns in Free-Ranging Sea Otters (*Enhydra lutris*) from British Columbia, Canada. *Environ. Toxicol. Chem.* **2011**, *30*, 2184−2193.
(43) Sciancalepore, G.; Pietroluongo, G.; Centelleghe, C.; Milan, M.; Bonato, M.; Corazzola, G.; Mazzariol, S. Evaluation of Per- and Poly-Fluorinated Alkyl Substances (PFAS) in Livers of Bottlenose Dolphins (*Tursiops truncatus*) Found Stranded along the Northern Adriatic Sea. *Environ. Pollut.* **2021**, *291*, 118186.
(44) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Bioconcentration and Tissue Distribution of Perfluorinated Acids in

Rainbow Trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **2003**, *22*, 196−204.
(45) United Nations Environment Programme Risk Profile of Perfluorooctane Sulfonate. *Rev. Lit. Arts Am.* **2006**, *34*.
(46) Ross, P. S.; Ellis, G. M.; Ikonomou, M. G.; Barrett-Lennard, L. G.; Addison, R. F. High PCB Concentrations in Free-Ranging Pacific Killer Whales, Orcinus orca: Effects of Age, Sex and Dietary Preference. *Mar. Pollut. Bull.* **2000**, *40*, 504−515.
(47) Reijnders, P. J. H.; Aguilar, A.; Borrell, A. Pollution and Marine Mammals. *Bull. World Health Organ.* **2007**, *85*, 890−898.
(48) Cullon, D. L.; Yunker, M. B.; Alleyne, C.; Dangerfield, N. J.; O'Neill, S.; Whiticar, M. J.; Ross, P. S. Persistent Organic Pollutants in Chinook Salmon (*Oncorhynchus tshawytscha*): Implications for Resident Killer Whales of British Columbia and Adjacent Waters. *Environ. Toxicol. Chem.* **2009**, *28*, 148−161.
(49) Debier, C.; Chalon, C.; Le Bœuf, B. J.; de Tillesse, T.; Larondelle, Y.; Thomé, J. P. Mobilization of PCBs from Blubber to Blood in Northern Elephant Seals (*Mirounga angustirostris*) during the Post-Weaning Fast. *Aquat. Toxicol.* **2006**, *80*, 149−157.
(50) Klosterhaus, S. L.; Grace, R.; Hamilton, M. C.; Yee, D. Method Validation and Reconnaissance of Pharmaceuticals, Personal Care Products, and Alkylphenols in Surface Waters, Sediments, and Mussels in an Urban Estuary. *Environ. Int.* **2013**, *54*, 92−99.
(51) David, A.; Fenet, H.; Gomez, E. Alkylphenols in Marine Environments: Distribution Monitoring Strategies and Detection Considerations. *Mar. Pollut. Bull.* **2009**, *58*, 953−960.
(52) Zoller, U. Estuarine and Coastal Zone Marine Pollution by the Nonionic Alkylphenol Ethoxylates Endocrine Disrupters: Is There a Potential Ecotoxicological Problem? *Environ. Int.* **2006**, *32*, 269−272.
(53) Jobling, S.; Sheahan, D.; Osborne, J. A.; Matthiessen, P.; Sumpter, J. P. Inhibition of Testicular Growth in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Estrogenic Alkylphenolic Chemicals. *Environ. Toxicol. Chem.* **1996**, *15*, 1942−2202.
(54) Diehl, J.; Johnson, S. E.; Xia, K.; West, A.; Tomanek, L. The Distribution of 4-Nonylphenol in Marine Organisms of North American Pacific Coast Estuaries. *Chemosphere* **2012**, *87*, 490−497.
(55) Gehring, M.; Vogel, D.; Tennhardt, L.; Weltin, D.; Bilitewski, B. *Bisphenol a Contamination of Wastepaper, Cellulose and Recycled Paper Products*Popov, V., Itoh, H., Brebbia, C. A., Kungolos, S., Eds.; Southampton: Boston, MA, USA, 2004; Vol. *78*.
(56) Soto, A. M.; Justicia, H.; Wray, J. W.; Sonnenschein, C. p-Nonyl-phenol: an estrogenic xenobiotic released from ″modified″ polystyrene. *Environ. Health Perspect.* **1991**, *92*, 167−173.
(57) Acir, I. H.; Guenther, K. Endocrine-Disrupting Metabolites of Alkylphenol Ethoxylates − A Critical Review of Analytical Methods, Environmental Occurrences, Toxicity, and Regulation. *Sci. Total Environ.* **2018**, *635*, 1530−1546.
(58) Sharma, M.; Chadha, P.; Madhu Sharma, C. Toxicity of Non-Ionic Surfactant 4-Nonylphenol an Endocrine Disruptor: A Review. *Int. J. Fish. Aquat. Stud.* **2018**, *6*, 190−197.
(59) Lam, J. C. W.; Lau, R. K. F.; Murphy, M. B.; Lam, P. K. S. Temporal Trends of Hexabromocyclododecanes (HBCDs) and Polybrominated Diphenyl Ethers (PBDEs) and Detection of Two Novel Flame Retardants in Marine Mammals from Hong Kong, South China. *Environ. Sci. Technol.* **2009**, *43*, 6944−6949.
(60) Marsh, G.; Athanasiadou, M.; Athanassiadis, I.; Bergman, Å.; Endo, T.; Haraguchi, K. Identification, Quantification, and Synthesis of a Novel Dimethoxylated Polybrominated Biphenyl in Marine Mammals Caught off the Coast of Japan. *Environ. Sci. Technol.* **2005**, *39*, 8684−8690.
(61) Zegers, B. N.; Mets, A.; van Bommel, R.; Minkenberg, C.; Hamers, T.; Kamstra, J. H.; Pierce, G. J.; Boon, J. P. Levels of Hexabromocyclododecane in Harbor Porpoises and Common Dolphins from Western European Seas, with Evidence for Stereoisomer-Specific Biotransformation by Cytochrome P450. *Environ. Sci. Technol.* **2005**, *39*, 2095−2100.
(62) Yang, C.; Abdallah, M. A. E.; Desborough, J.; Burniston, D.; Tomy, G.; Harrad, S.; Marvin, C. Trends in Hexabromocyclododecanes in the UK and North America. *Sci. Total Environ.* **2019**, *658*, 861−867.
(63) Stapleton, H. M.; Dodder, N. G.; Kucklick, J. R.; Reddy, C. M.; Schantz, M. M.; Becker, P. R.; Gulland, F.; Porter, B. J.; Wise, S. A. Determination of HBCD, PBDEs and MeO-BDEs in California Sea Lions (*Zalophus californianus*) Stranded between 1993 and 2003. *Mar. Pollut. Bull.* **2006**, *52*, 522−531.
(64) Fair, P. A.; Lee, H. B.; Adams, J.; Darling, C.; Pacepavicius, G.; Alaee, M.; Bossart, G. D.; Henry, N.; Muir, D. Occurrence of Triclosan in Plasma of Wild Atlantic Bottlenose Dolphins (*Tursiops truncatus*) and in Their Environment. *Environ. Pollut.* **2009**, *157*, 2248−2254.
(65) Bennett, E. R.; Ross, P. S.; Huff, D.; Alaee, M.; Letcher, R. J. Chlorinated and Brominated Organic Contaminants and Metabolites in the Plasma and Diet of a Captive Killer Whale (*Orcinus orca*). *Mar. Pollut. Bull.* **2009**, *58*, 1078−1083.
(66) Singer, H.; Müller, S.; Tixier, C.; Pillonel, L. Triclosan: Occurrence and Fate of a Widely Used Biocide in the Aquatic Environment: Field Measurements in Wastewater Treatment Plants, Surface Waters, and Lake Sediments. *Environ. Sci. Technol.* **2002**, *36*, 4998−5004.
(67) Boyd, G. R.; Reemtsma, H.; Grimm, D. A.; Mitra, S. Pharmaceuticals and Personal Care Products (PPCPs) in Surface and Treated Waters of Louisiana, USA and Ontario, Canada. *Sci. Total Environ.* **2003**, *311*, 135−49.
(68) Lee, J. D.; Lee, J. Y.; Kwack, S. J.; Shin, C. Y.; Jang, H. J.; Kim, H. Y.; Kim, M. K.; Seo, D. W.; Lee, B. M.; Kim, K. B. Risk Assessment of Triclosan, a Cosmetic Preservative. *Toxicol. Res.* **2019**, *35*, 137−154.
(69) Bossi, R.; Riget, F. F.; Dietz, R.; Sonne, C.; Fauser, P.; Dam, M.; Vorkamp, K. Preliminary Screening of Perfluorooctane Sulfonate (PFOS) and Other Fluorochemicals in Fish, Birds and Marine Mammals from Greenland and the Faroe Islands. *Environ. Pollut.* **2005**, *136*, 323−329.
(70) Houde, M.; Balmer, B. C.; Brandsma, S.; Wells, R. S.; Rowles, T. K.; Solomon, K. R.; Muir, D. C. G. Perfluoroalkyl Compounds in Relation to Life-History and Reproductive Parameters in Bottlenose Dolphins (*Tursiops truncatus*) from Sarasota Bay, Florida, USA. *Environ. Toxicol. Chem.* **2006**, *25*, 2405−2412.
(71) Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J. F.; Hansen, K. J.; Jones, P. D.; Helle, E.; Nyman, M.; Giesy, J. P. Accumulation of Perfluorooctane Sulfonate in Marine Mammals. *Environ. Sci. Technol.* **2001**, *35*, 1593−1598.
(72) Spaan, K. M.; van Noordenburg, C.; Plassmann, M. M.; Schultes, L.; Shaw, S.; Berger, M.; Heide-Jørgensen, M. P.; Rosing-Asvid, A.; Granquist, S. M.; Dietz, R.; Sonne, C.; Rigét, F.; Roos, A.; Benskin, J. P. Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere. *Environ. Sci. Technol.* **2020**, *54*, 4046−4058.
(73) Wang, Q.; Ruan, Y.; Jin, L.; Zhang, X.; Li, J.; He, Y.; Wei, S.; Lam, J. C. W.; Lam, P. K. S. Target, Nontarget, and Suspect Screening and Temporal Trends of Per- And Polyfluoroalkyl Substances in Marine Mammals from the South China Sea. *Environ. Sci. Technol.* **2021**, *55*, 1045−1056.
(74) Zhang, B.; He, Y.; Yang, G.; Chen, B.; Yao, Y.; Sun, H.; Kannan, K.; Zhang, T. Legacy and Emerging Poly- And Perfluoroalkyl Substances in Finless Porpoises from East China Sea: Temporal Trends and Tissue-Specific Accumulation. *Environ. Sci. Technol.* **2021**, *56*, 623.
(75) Stephens, N.; Gaylard, S.; Holyoake, C.; Duignan, P.; O'Dea, M.; Warren, K. Per and Polyfluorinated Alkyl Substances (PFAS) in Coastal and Estuarine Indo-Pacific Bottlenose Dolphins (*Tursops aduncus*), Western Australia (WA). In *Paper Presented at the World Marine Mammal Conference*: Barcelona, Spain, 2019.
(76) Harris, M. F.; Logan, J. L. Determination of Log Kow Values for Four Drugs. *J. Chem. Educ.* **2014**, *91*, 915−918.
(77) Alava, J. J.; Lambourn, D.; Olesiuk, P.; Lance, M.; Jeffries, S. J.; Gobas, F. A. P. C.; Ross, P. S. PBDE Flame Retardants and PCBs in

https://doi.org/10.1021/acs.est.2c04126
Environ. Sci. Technol. 2023, 57, 360−374

Migrating Steller Sea Lions (*Eumetopias jubatus*) in the Strait of Georgia, British Columbia, Canada. *Chemosphere* **2012**, *88*, 855−864.
(78) Desforges, J. P. W.; Ross, P. S.; Loseto, L. L. Transplacental Transfer of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Arctic Beluga Whales (*Delphinapterus leucas*). *Environ. Toxicol. Chem.* **2012**, *31*, 296−300.
(79) Greig, D. J.; Ylitalo, G. M.; Hall, A. J.; Fauquier, D. A.; Gulland, F. M. D. Transplacental Transfer of Organochlorines in California Sea Lions (*Zalophus californianus*). *Environ. Toxicol. Chem.* **2007**, *26*, 37−44.
(80) Ding, N.; Harlow, S. D.; Randolph Jr, J. F.; Loch-Caruso, R.; Park, S. K. Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) and Their Effects on the Ovary. *Hum. Reprod. Update* **2020**, *26*, 724−752.
(81) Conder, J. M.; Hoke, R. A.; Wolf, W.; Russell, M. H.; Buck, R. C. Are PFCAs Bioaccumulative? A Critical Review and Comparison with Regulatory Criteria and Persistent Lipophilic Compounds. *Environ. Sci. Technol.* **2008**, *42*, 995−1003.
(82) Hart, K.; Kannan, K.; Isobe, T.; Takahashi, S.; Yamada, T. K.; Miyazaki, N.; Tanabe, S. Time Trends and Transplacental Transfer of Perfluorinated Compounds in Melon-Headed Whales Stranded along the Japanese Coast in 1982, 2001/2002, and 2006. *Environ. Sci. Technol.* **2008**, *42*, 7132−7137.
(83) Shaw, S.; Berger, M. L.; Brenner, D.; Tao, L.; Wu, Q.; Kannan, K. Specific Accumulation of Perfluorochemicals in Harbor Seals (*Phoca vitulina concolor*) from the Northwest Atlantic. *Chemosphere* **2009**, *74*, 1037−1043.
(84) Taylor, S.; Terkildsen, M.; Stevenson, G.; de Araujo, J.; Yu, C.; Yates, A.; McIntosh, R. R.; Gray, R. Per and Polyfluoroalkyl Substances (PFAS) at High Concentrations in Neonatal Australian Pinnipeds. *Sci. Total Environ.* **2021**, *786*, 147446.
(85) Grønnestad, R.; Villanger, G. D.; Polder, A.; Kovacs, K. M.; Lydersen, C.; Jenssen, B. M.; Borgå, K. Maternal Transfer of Perfluoroalkyl Substances in Hooded Seals. *Environ. Toxicol. Chem.* **2017**, *36*, 763−770.
(86) Midasch, O.; Drexler, H.; Hart, N.; Beckmann, M. W.; Angerer, J. Transplacental Exposure of Neonates to Perfluorooctanesulfonate and Perfluorooctanoate: A Pilot Study. *Int. Arch. Occup. Environ. Health* **2007**, *80*, 643−648.
(87) Chen, M. L.; Chang, C. C.; Shen, Y. J.; Hung, J. H.; Guo, B. R.; Chuang, H. Y.; Mao, I. F. Quantification of Prenatal Exposure and Maternal-Fetal Transfer of Nonylphenol. *Chemosphere* **2008**, *73*, 91.
(88) Tan, B. L. L.; Mohd, M. A. Analysis of Selected Pesticides and Alkylphenols in Human Cord Blood by Gas Chromatograph-Mass Spectrometer. *Talanta* **2003**, *61*, 385−391.
(89) Li, L. X.; Chen, L.; Meng, X. Z.; Chen, B. H.; Chen, S. Q.; Zhao, Y.; Zhao, L. F.; Liang, Y.; Zhang, Y. H. Exposure Levels of Environmental Endocrine Disruptors in Mother-Newborn Pairs in China and Their Placental Transfer Characteristics. *PLoS One* **2013**, *8*, No. e62526.
(90) Griffiths, S. K.; Campbell, J. P. Placental Structure, Function and Drug Transfer. *Contin. Educ. Anaesthesia, Crit. Care Pain* **2015**, *15*, 84−89.
(91) Alava, J. J.; Ross, P. S.; Lachmuth, C.; Ford, J. K. B.; Hickie, B. E.; Gobas, F. A. P. C. Habitat-Based PCB Environmental Quality Criteria for the Protection of Endangered Killer Whales (*Orcinus orca*). *Environ. Sci. Technol.* **2012**, *46*, 12655−12663.
(92) Huang, M. C.; Robinson, V. G.; Waidyanatha, S.; Dzierlenga, A. L.; DeVito, M. J.; Eifrid, M. A.; Gibbs, S. T.; Blystone, C. R. Toxicokinetics of 8:2 Fluorotelomer Alcohol (8:2-FTOH) in Male and Female Hsd:Sprague Dawley SD Rats after Intravenous and Gavage Administration. *Toxicol. Reports* **2019**, *6*, 924−932.
(93) Butt, C. M.; Muir, D. C. G.; Mabury, S. A. Biotransformation Pathways of Fluorotelomer-Based Polyfluoroalkyl Substances: A Review. *Environ. Toxicol. Chem.* **2014**, *33*, 243−267.
(94) Loewen, M.; Halldorson, T.; Wang, F.; Tomy, G. Fluorotelomer Carboxylic Acids and PFOS in Rainwater from an Urban Center in Canada. *Environ. Sci. Technol.* **2005**, *39*, 2944−2951.
(95) Alava, J. J.; Ross, P. S.; Gobas, F. A. P. C. Food Web Bioaccumulation Model for Resident Killer Whales from the Northeastern Pacific Ocean as a Tool for the Derivation of PBDE-Sediment Quality Guidelines. *Arch. Environ. Contam. Toxicol.* **2016**, *70*, 155−168.
(96) Desforges, J. P. W.; Hall, A.; McConnell, B.; Rosing-Asvid, A.; Barber, J. L.; Brownlow, A.; De Guise, S.; Eulaers, I.; Jepson, P. D.; Letcher, R. J.; Levin, M.; Ross, P. S.; Samarra, F.; Víkingson, G.; Sonne, C.; Dietz, R. Predicting Global Killer Whale Population Collapse from PCB Pollution. *Science (80-.)* **2018**, *361*, 1373−1376.
(97) Rayne, S.; Ikonomou, M. G.; Ross, P. S.; Ellis, G. M.; Barrett-Lennard, L. G. PBDEs, PBBs, and PCNs in Three Communities of Free-Ranging Killer Whales (*Orcinus orca*) from the Northeastern Pacific Ocean. *Environ. Sci. Technol.* **2004**, *38*, 4293−4299.
(98) Johnson, L. L.; Ylitalo, G. M.; Arkoosh, M. R.; Kagley, A. N.; Stafford, C.; Bolton, J. L.; Buzitis, J.; Anulacion, B. F.; Collier, T. K. Contaminant Exposure in Outmigrant Juvenile Salmon from Pacific Northwest Estuaries of the United States. *Environ. Monit. Assess.* **2007**, *124*, 167−194.
(99) Ross, P. S.; Jeffries, S. J.; Yunker, M. B.; Addison, R. F.; Ikonomou, M. G.; Calambokidis, J. C. Harbor Seals (*Phoca vitulina*) in British Columbia, Canada, and Washington State, USA, Reveal a Combination of Local and Global Polychlorinated Biphenyl, Dioxin, and Furan Signals. *Environ. Toxicol. Chem.* **2004**, *23*, 157−165.
(100) Buckman, A. H.; Veldhoen, N.; Ellis, G.; Ford, J. K. B.; Helbing, C. C.; Ross, P. S. PCB-Associated Changes in MRNA Expression in Killer Whales (*Orcinus orca*) from the NE Pacific Ocean. *Environ. Sci. Technol.* **2011**, *45*, 10194−10202.
(101) Mos, L.; Cameron, M.; Jeffries, S. J.; Koop, B. F.; Ross, P. S. Risk-Based Analysis of Polychlorinated Biphenyl Toxicity in Harbor Seals. *Integr. Environ. Assess. Manag.* **2010**, *6*, 631−640.
(102) Fair, P. A.; Romano, T.; Schaefer, A. M.; Reif, J. S.; Bossart, G. D.; Houde, M.; Muir, D.; Adams, J.; Rice, C.; Hulsey, T. C.; Peden-Adams, M. Associations between Perfluoroalkyl Compounds and Immune and Clinical Chemistry Parameters in Highly Exposed Bottlenose Dolphins (*Tursiops truncatus*). *Environ. Toxicol. Chem.* **2013**, *32*, 736−746.
(103) Huggins, J. L.; Garner, M. M.; Raverty, S. A.; Lambourn, D. M.; Norman, S. A.; Rhodes, L. D.; Gaydos, J. K.; Olson, J. K.; Haulena, M.; Hanson, M. B. The Emergence of Mucormycosis in Free-Ranging Marine Mammals of the Pacific Northwest. *Front. Mar. Sci.* **2020**, *7*, 555.
(104) Ojo, A. F.; Peng, C.; Ng, J. C. Assessing the Human Health Risks of Per- and Polyfluoroalkyl Substances: A Need for Greater Focus on Their Interactions as Mixtures. *J. Hazard. Mater.* **2021**, *407*, 124863.
(105) *Current Therapy in Large Animal Theriogenology*, 2nd ed.; Youngquist, R. S., Threlfall, W. R., Eds.; Saunders Elsevier, 2007.
(106) Jacobson, C.; Bruce, M.; Kenyon, P.; Lockwood, A.; Miller, D.; Refshauge, G.; Masters, D. *A Review of Maternal Dystocia*; North Sydney, 2020.

https://doi.org/10.1021/acs.est.2c04126
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Supporting Information

Emerging Contaminants and New POPs (PFAS and HBCDD) in Endangered Southern Resident and Bigg's (Transient) Killer Whales (*Orcinus orca*): *In Utero* Maternal Transfer and Pollution Management Implications

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Further information on the analyzed 'contaminants of emerging concern' (CECs) and new persistent organic pollutants (POPs)

Alkylphenols (APs) are commonly detected in the ocean and attributed to anthropogenic activities. As many countries have not yet implemented adequate removal and monitoring measures, these pollutants are mainly discharged to the ocean through sewage treatment plants and industry effluent[1–3]. This class of compounds includes alkylphenol ethoxylates (APEOs) which are primarily incorporated into herbicides, pesticides, lubricating oils, and surfactants such as detergents, wetting agents, and dispersants[4]. Approximately 80% of AEPOs are nonylphenol polyethoxylates with the remainder consisting of octylphenol and dodecylephenol ethoxylates. These compounds biodegrade in sewage treatment processes to nonylphenols (NPs), octylphenols, and other mono-, di-, and tri-ethoxylates that are then released into the environment[1]. The daughter compounds of APs are not readily degradable and are more toxic and persistent (particularly NPs). They have strong hydrophilic and hydrophobic properties, are low in solubility, and tend to partition with organic matter[2,3].

Although the toxic effects of AEPOs are well documented, few studies on the effects of AEPOs in marine mammals have been published. This contaminant is predominant in the surface layer of the ocean where it can interact with the biota and become incorporated into the food chain[1]. In rats and humans, APs have been shown to disrupt endocrine systems (particularly estrogen production) and impact developmental and reproductive success[1,5]. AEPOs also have the ability to interact with the nervous system and influence cognitive functions (NPs were especially shown to impact the development of dendritic and synaptic cells), inflammation, cell damage, and apoptosis in humans[3]. In fish, invertebrates, and mice, these compounds are estrogenic and in humans, exposure to AEPOs can impact T cell expression[3,6,7].

Hexabromocyclododecane (HBCDD) is a brominated flame retardant that is extensively used and persists in the environment[8]. In 2001 there was a significant increase in the global demand for this chemical that is commonly used in the manufacture of clothing, building insulation, furniture textiles, and electrical equipment[9,10]. Because HBCDD compounds do not readily chemically bind to materials, it is readily released into the environment through weathering and degradation. Similar to APs, HBCDD is lipophilic, persists in the ocean, and bioaccumulates through the food web, impacting those marine mammals found at higher trophic levels[11]. While few studies have looked at the toxic implications of HBCDDs on wildlife, research has claimed that exposure may be linked to various long term effects. Among these are disruption of thyroid homeostasis, decreased biotransformation of enzyme activity, and neurobehavioral alterations[12,13].

Triclosan (2,4,4′-trichloro-2 2′-hydroxydiphenyl ether) is a pharmaceutical and personal care product (PPCP) pollutant that has only recently been recognized as a concern in marine ecosystems. It is prevalent throughout society as it is an antibacterial compound commonly found in consumer products like toothpaste, soaps, detergents, toys, textile fabrics and cleaning products. This chemical is mainly released into the ocean through residential wastewater and sewage effluent[14,15]. Although studies suggest triclosan can be photolytically degraded to 2,8-dichlorodibenzo-p-dioxin (DCDD) within 3 days of its release into the photic zone of the ocean, others investigations have shown that it may transform to more persistent and toxic forms such as methyl-triclosan, chlorophenols, and chlorinated dioxins[16–19]. Methyl-triclosan is likely formed through biological methylation of triclosan and released into the marine environment through WWTPs[20,21]. Although both contaminants are a concern to marine ecosystem health, methyl-triclosan is more persistent, and has a stronger propensity to accumulate in fatty tissues

and biomagnify throughout the food web. Studies have documented that triclosan and methyl-triclosan can disrupt fatty acid production, increase microbial resistance, impair endocrine system, and decrease reproductive success in lower level food chain organisms[16,17,20,22].

Per- and polyfluoroalkyl substances (PFAS) are a subset of organofluorine compounds that are exceptionally persistent in the environment and are widely used in industrial settings[23,24]. These chemicals have a composition that is extremely stable, and are both hydrophilic and lipophilic, allowing for their frequent application in food packaging, stain and water repellent fabrics, cooking ware, paints, and fire extinguisher foam. As they have a low detection threshold within the marine environment, their prevalence throughout the environment has only recently been recognized[23,25,26]. Like the previously discussed contaminants, PFAS can bioaccumulate throughout the food chain; however, they are known to bind to and concentrate in protein rich tissues such as blood, skeletal muscles, and liver. Studies suggest that they abrogate intracellular communication in in-vitro dolphin kidney epithelial cells, and contribute to hepatocellular damage in fish[27–29].

Additional method details

Tissue sampling and additional data collection

In BC, distressed, moribund, and dead free floating or beach cast killer whales are reported by the public, Indigenous communities, biologists, and research scientists to the British Columbia Marine Mammal Stranding Network. This triggers the mobilization of a response team. For dead floating whales, animals were secured and towed ashore, typically at high tides along secluded or secured beaches with access for necropsy during receding and low tides. Morphometrics and tissue sampling were performed according to standardized necropsy protocols[30]. Animals were initially photographed for identification, then morphometrics were compiled and the stranding

location, date, sex, ecotype, age, class, and carcass condition code (CC) were recorded[31]. Between 2006 and 2018 twelve dead whales were reported along the coast of BC (Figure S1); necropsies and collection of tissue specimens was performed on these individuals (excluding L98 [Luna]: case 06/00938) following systematic gross necropsies according to established protocols[31]. Representative samples were harvested and preserved in formalin for histopathology and a suite of fresh tissues (including skeletal muscle (SM) and liver samples used in the present study) were either wrapped in aluminum foil or placed in labelled plastic bags, chilled on wet ice, and transported to a diagnostic laboratory. The list of tissues sampled in the necropsies is well detailed in *Raverty et al.*[30]. The tissues were then subsampled and forwarded for diagnostic studies while legacy samples were frozen at -80°C. A tissue inventory, sample disposition, and test results were recorded in an excel spreadsheet.

Analytical methods

Alkylphenols (APs; laboratory procedure MLA-080 Rev 02 Ver 04, SGS AXYS Analytical Services Ltd.). AP concentrations were obtained by preparation of a solution of up to 2g wet weight (ww) of liver or skeletal muscle in water spiked with isotopically labelled surrogate standards, $^{13}C_6$-4-nonylphenol and $^{13}C_6$-4-nonylphenol diethoxylate. Samples were extracted by exhaustive steam distillation with concurrent liquid-liquid extraction using isooctane. Resulting extracts were cleaned up by solid phase extraction (SPE) using disposable cartridges containing aminopropyl sorbent. The SPE eluate was prepared in methanol, spiked with recovery standards and analyzed on a high performance liquid chromatography reversed phase C18 column using a solvent gradient which was coupled to a triple quadrupole mass spectrometer run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode. The sample extracts were analyzed in two separate liquid chromatography/mass spectrometry (LC-

MS/MS) runs, one run in the ESI negative mode (for nonyl-phenol and n-octyl-phenol), and the other run in the ESI positive mode (for NP1EO and NP2EO). Peak areas in the sample chromatography are converted to concentrations using the average relative response factor (RRF) and are determined with respect to the appropriate labelled surrogate. Average relative response factors (RRF) are determined from a bracketing calibration involving known amounts of native, surrogate and recovery compounds.

Hexabromocyclododecane (HBCDD; laboratory procedure MLA-070 Rev 02 Ver 05, SGS AXYS Analytical Services Ltd.). To determine the concentration of alpha-, beta-, and gamma-HBCDD, samples (up to 10g ww) were initially spiked with isotopically labelled surrogate standards(^{13}C-alpha-, ^{13}C-beta-, and ^{13}C-gamma-HBCDD) then Soxhlet extracted with dichloromethane. Florisil and BioBead columns were used for cleanup purposes. The final extracts were analyzed on a high or ultrahigh performance liquid chromatography reversed phase C18 column using a solvent gradient which was coupled to a triple quadrupole mass spectrometer run at unit mass resolution in the MRM mode. Calibration for this instrument was performed using a series of standard solutions containing known amounts of native, surrogate and recovery compounds. Target compounds were quantified using the isotope dilution/internal standard method which involved comparing the area of the quantification ion to that of the ^{13}C-labelled standards and correcting for relative response factors (RRFs).

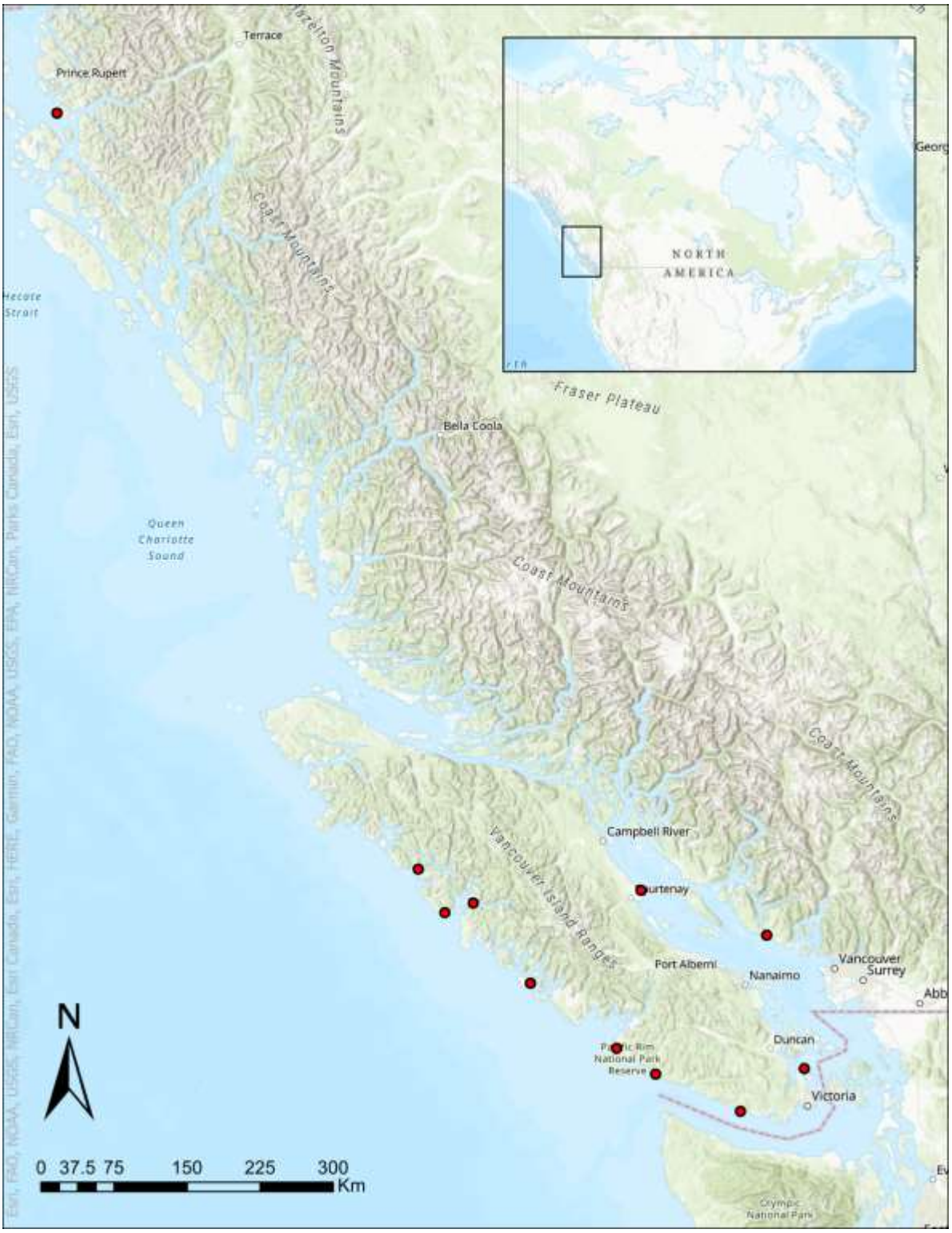
Triclosan and Methyl Triclosan (laboratory procedure MLA-115 Rev 01 Ver 02, SGS AXYS Analytical Services Ltd.). To determine triclosan and methyl triclosan concentrations, samples (up to 5 g ww) were first spiked with isotopically labelled surrogate standards and processed by Soxhlet extraction. The extracts were cleaned by gel permeation chromatography

and derivatized with acetic anhydride before a final clean up on a florisil column. After being spiked with an internal standard, the extracts were analyzed by capillary gas chromatography coupled with a high-resolution mass spectrometer (with a DB-5 capillary column) that was operated at a static (8000) mass resolution (10% valley) in the electron ionization (EI) mode using multiple ion detection (MID) to obtain two characteristic ions for each target analyte and surrogate standard. Calibration of this instrument was performed by derivatized calibration solutions containing native target analytes, labelled surrogates, and recovery standards. Target compounds were quantified using the isotope dilution/internal standard method which involved comparing the area of the quantification ion to that of the labelled surrogate standards and correcting for relative response factors (RRFs).

Per- and polyfluoroalkyl substances (PFAS; laboratory procedure MLA 110 Rev 02 Ver 11, SGS AXYS Analytical Services Ltd.). PFAS concentrations were determined by spiking up to 2g ww samples with isotopically labeled surrogate standards, and performing three consecutive extractions using methanolic potassium hydroxide solution, acetonitrile, and methanolic potassium hydroxide solution, respectively. The supernatant was collected with each extraction and combined to create one extract per sample. The combined extracts were treated with ultra-pure carbon powder and evaporated to remove methanol. This extract was then diluted with water and cleaned by solid phase extraction (SPE) using disposable cartridges containing a weak anion exchange sorbent. Extracts were spiked with recovery standards and analyzed by ultrahigh performance liquid chromatography (UPLC-MS/MS) reversed phase C18 column using a solvent gradient. This analysis was coupled to a triple quadrupole mass spectrometer run at unit mass resolution in the MRM mode in negative electrospray ionization mode. It is important to note that 7:3 FTCA had 2 MRMs, which must meet a ratio criteria for positive

identification of 7:3 FTCAs. Calibration of the UPLC-MS/MS instrument was performed by the analysis of at least five calibration solutions. Target compounds were quantified using the isotope dilution/internal standard method which involved comparing the area of the quantification ion to that of the labelled surrogate standards and correcting for relative response factors (RRFs).

Quality Assurance/Quality Control (QA/QC). The analysis of CECs and new POPs followed the Quality Control Acceptance Criteria of SGS AXYS Analytical Services. Tissue samples were analyzed in batches consisting of a maximum of 20 samples, with one procedural blank and one spiked matrix (OPR) sample for quality assurance and quality controls (QC) per batch. A clean reference tissue was used as the matrix for batch QC samples. A duplicate was analyzed, provided there were sufficient samples. The batch was carried through the complete analytical process as a unit. Additional QC parameters were followed according to the individual methods for additional parameters such as mass Calibration verification, retention time (RT) window, instrument sensitivity check (ISC), instrument background and instrument carryover. Reporting limits were provided for each method but depending on the method and the analyte, the reporting limit was dictated by a different lower limit; HBCDD was reported to the LMCL (lower method calibration level), APs were reported to a minimum level set by the method, triclosan was reported to SDLs (sample specific detection limits), and PFAS was reported to the greater of the minimum level, or the SDL.



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209 **Figure S1.** Stranding locations of the 12 killer whales (*Orcinus orca*) analyzed in the present

210 study along the coast of British Columbia, Canada, sampled from 2006 to 2018. Note: two

stranding locations are overlapping due to the mother-fetus pair (J32 Mother and J32 Fetus) of tissue samples that were collected together.

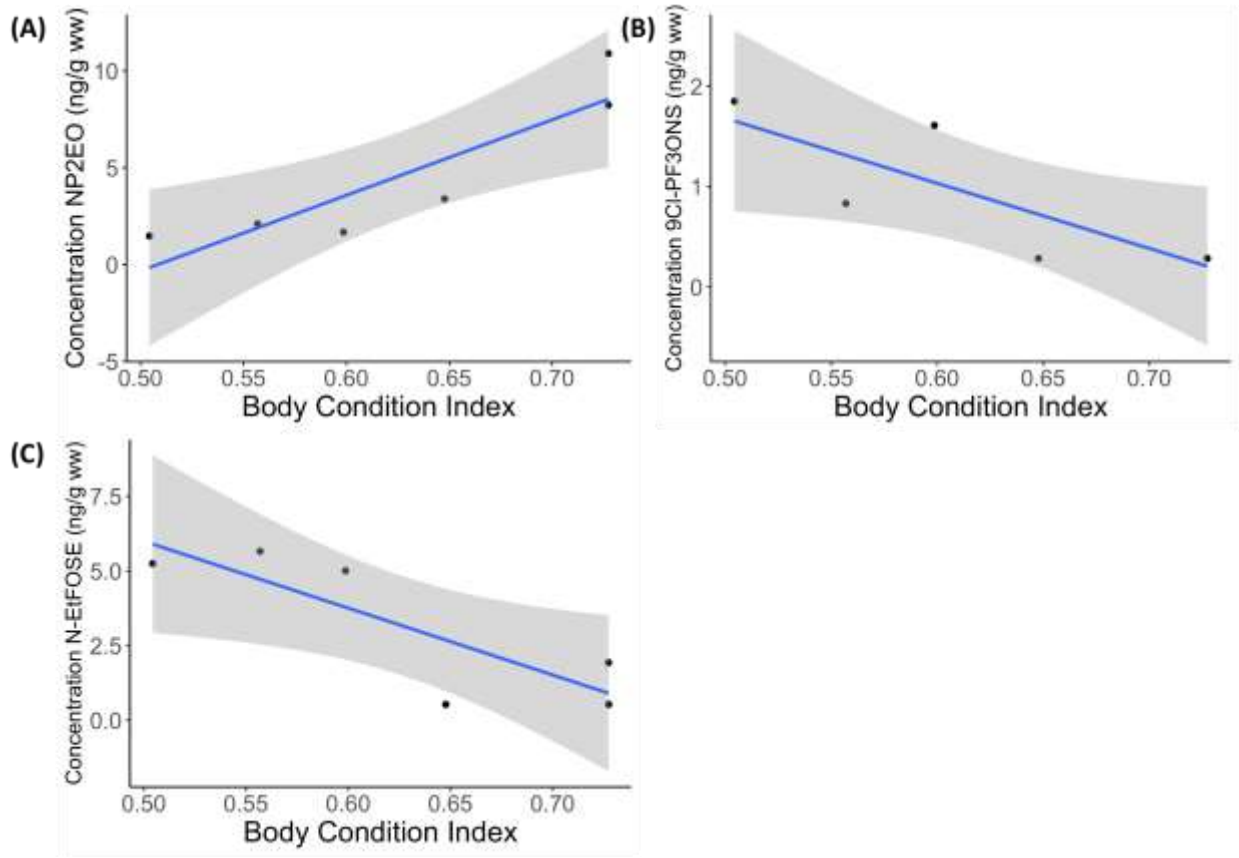
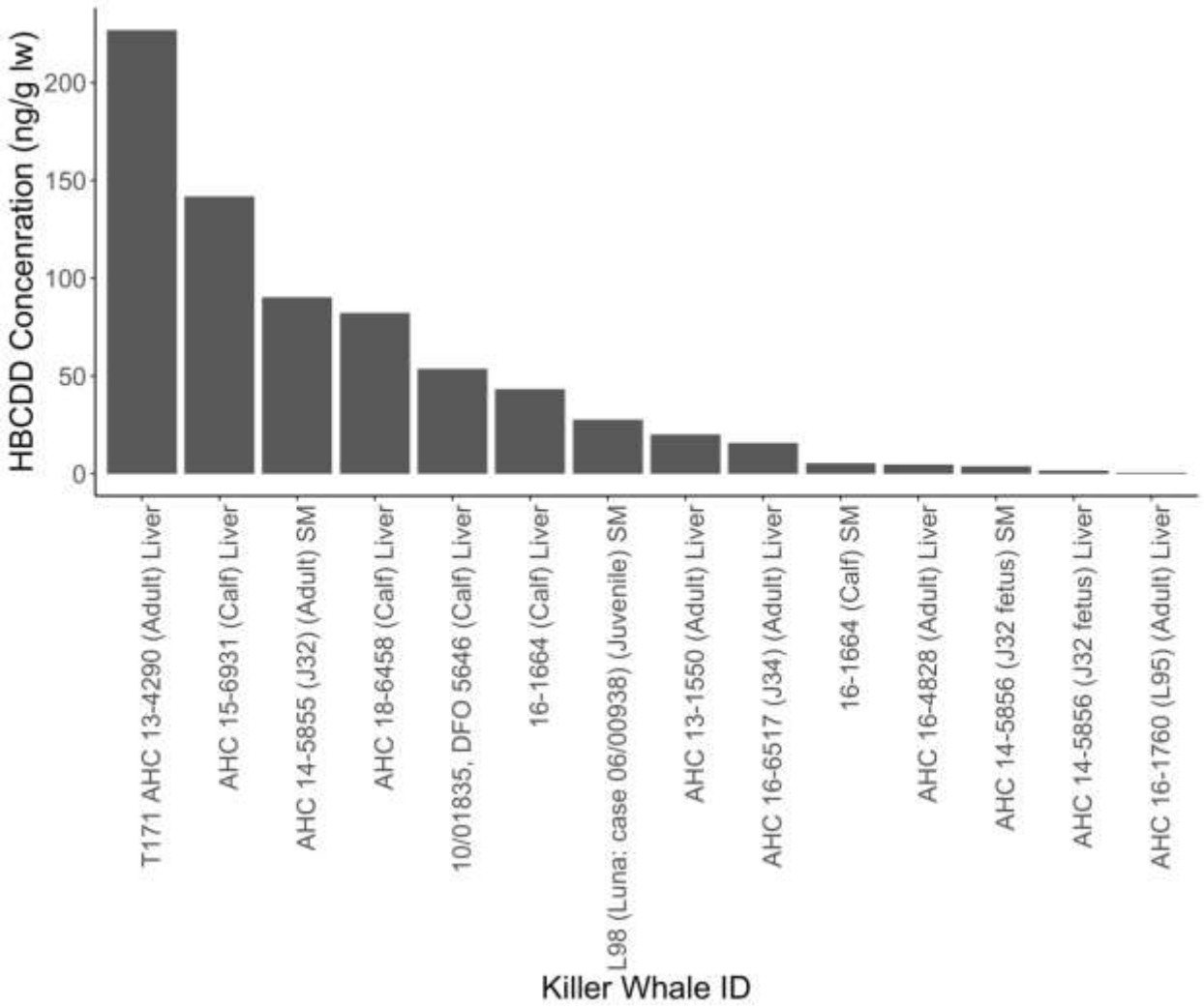


Figure S2. Significant correlations between body condition index and contaminant concentration (ng/g ww; NP2EO $p = 0.016$, $r = 0.89$; 9Cl-PF3ONS $p = 0.042$, $r = -0.83$; N-EtFOSE $p = 0.036$, $r = -0.84$). BCI is a function of the individual killer whale's girth and length (BCI=girth/length) and ranges from poor values (BCI = 0.5-0.6) to good (BCI = 0.6-0.7)[32]. Individuals that may have been artificially inflated to mimic bloating or pregnancy have a BCI ranging from 0.7-0.8. Best-fit lines are denoted in blue with 95% confidence levels shown in dark grey.



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222 **Figure S3.** Concentration (ng/g lipid weight (lw)) of HBCDD for each killer whale (*O. orca*)

223 sample (n=14, skeletal muscle (SM) and liver).

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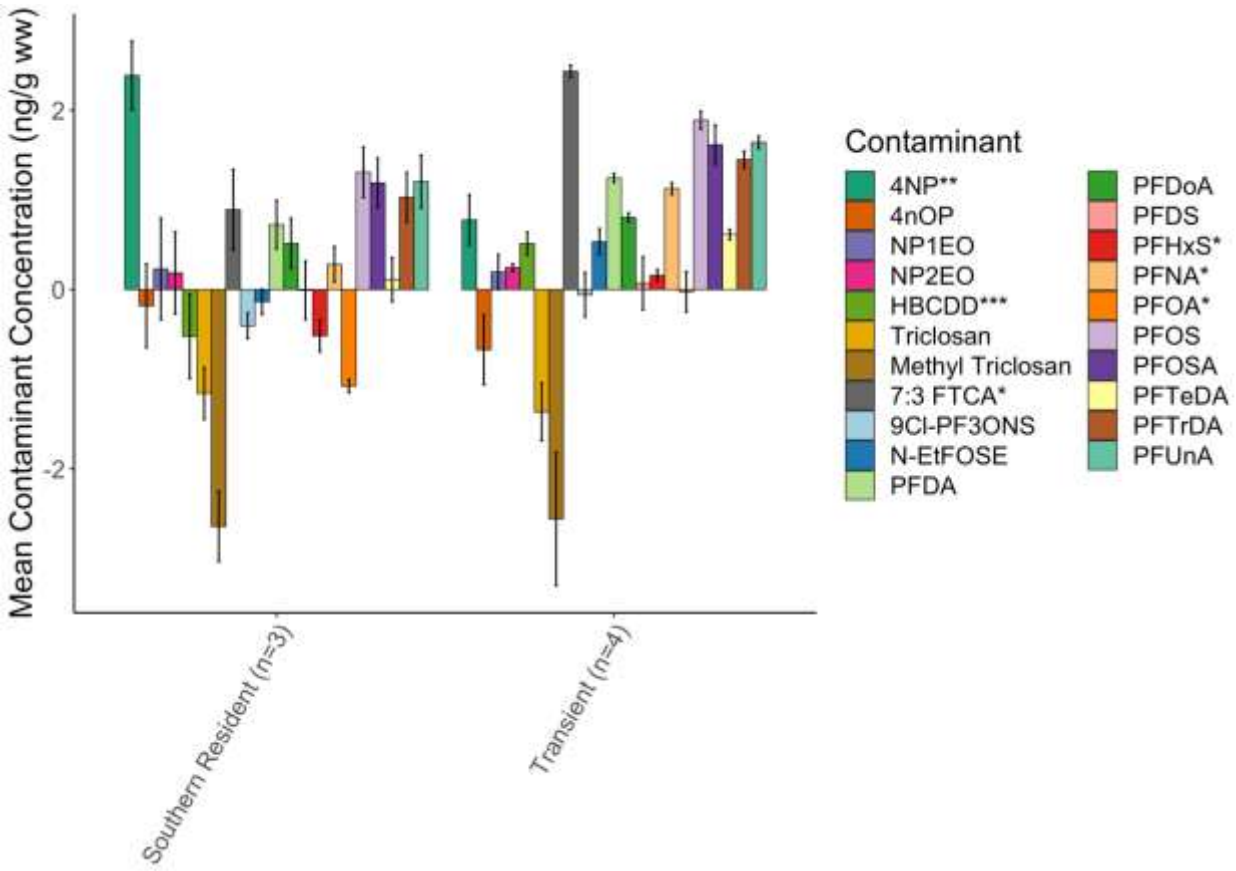

Figure S4. Difference in mean contaminant concentrations (ng/g ww) in Southern Resident (n=3) calves and Bigg's killer whale (*O. orca*) calves (n=4) skeletal muscle and liver samples. Contaminant data sets are presented in log scale and standard error bars are shown. * denotes those CEC and new POP concentrations significantly higher in Bigg's killer whale calves, while ** denotes those significantly higher in SRKW calves. *** HBCDD is presented in wet weight.

Table S1. Raw wet weight contaminant concentration (ng/g ww) for each killer whale (*O. orca*) sample (killer whale ID). Data is not blank corrected. SM = Skeletal Muscle, ND = Not Detected at reporting limit.

Excel sheet Table S1.

Table S2. Wet weight and lipid normalized contaminant concentration (ng/g) for each killer

whale (*O. orca*) sample (killer whale ID). SM = Skeletal muscle.

Excel sheet Table S2.

Table S3. Maternal transfer ratio (MTR) for each contaminant and its associated octanol-water partition coefficient (log K_{ow}). NA = Not Available.

Contaminant	log K_{ow}	Maternal Transfer Ratio (MTR)	Maternal Transfer Rates (%)[d]
4NP	5.76[33]	19.39	95.1
4nOP	5.56[34]	5.32	84.17
NP1EO	4.17[35]	1.73	63.36
NP2EO	4.21[35]	2.42	70.8
Methyl Triclosan	5.2[36]	1.64	62.07
Triclosan	4.76[33]	0.54	37.29
HBCDD	5.07[37]	0.093	8.52
7:3 FTCA	NA	1	50
9Cl-PF3ONS	5.01[38, a]	1	50
N-EtFOSE	6.52[39]	1	50
PFDA	6.5[39]	5.15	83.74
PFDoA	7.6[33, b]	4.95	83.19
PFDS	7.6[39]	3.39	77.24
PFHxS	5.17[39]	1.65	62.33
PFNA	5.92[39]	10.89	91.59
PFOA	5.3[39]	1	50
PFOS	6.43[39]	4.37	81.37
PFOSA	5.8[33, c]	2.81	73.76
PFTeDA	8.9[39]	3.02	75.12
PFTrDA	8.25[39]	5.11	83.64
PFUnA	7.15[39]	5.4	84.38

[a] Albumin/water partition coefficient
[b] XLogP3-AA
[c] Estimate
[d] Transfer rate (%) = contaminant concentration in J32 Fetus / (contaminant concentration in J32 Fetus + contaminant concentration in J32 Mother) * 100[40]. Note that contaminant burdens could not be calculated as total skeletal muscle mass measurements were not available.

References

(1) David, A.; Fenet, H.; Gomez, E. Alkylphenols in Marine Environments: Distribution Monitoring Strategies and Detection Considerations. *Mar. Pollut. Bull.* **2009**, *58* (7), 953–960. https://doi.org/10.1016/j.marpolbul.2009.04.021.

(2) Soares, A.; Guieysse, B.; Jefferson, B.; Cartmell, E.; Lester, J. N. Nonylphenol in the Environment: A Critical Review on Occurrence, Fate, Toxicity and Treatment in Wastewaters. *Environ. Int.* **2008**, *34* (7), 1033–1049. https://doi.org/10.1016/j.envint.2008.01.004.

(3) Acir, I. H.; Guenther, K. Endocrine-Disrupting Metabolites of Alkylphenol Ethoxylates – A Critical Review of Analytical Methods, Environmental Occurrences, Toxicity, and Regulation. *Sci. Total Environ.* **2018**, *635*, 1530–1546. https://doi.org/10.1016/j.scitotenv.2018.04.079.

(4) Shang, D. Y.; Macdonald, R. W.; Ikonomou, M. G. Persistence of Nonylphenol Ethoxylate Surfactants and Their Primary Degradation Products in Sediments from near a Municipal Outfall in the Strait of Georgia, British Columbia, Canada. *Environ. Sci. Technol.* **1999**, *33* (9), 1366–1372. https://doi.org/10.1021/es980966z.

(5) Soto, A. M.; Justicia, H.; Wray, J. W.; Sonnenschein, C. P-Nonyl-Phenol: An Estrogenic Xenobiotic Released from "modified" Polystyrene. *Environ. Health Perspect.* **1991**, *92* (Cd), 167–173. https://doi.org/10.1289/ehp.9192167.

(6) Sadakane, K.; Ichinose, T.; Takano, H.; Yanagisawa, R.; Koike, E.; Inoue, K. I. The Alkylphenols 4-Nonylphenol, 4-Tert-Octylphenol and 4-Tert-Butylphenol Aggravate Atopic Dermatitis-like Skin Lesions in NC/Nga Mice. *J. Appl. Toxicol.* **2014**, *34* (8), 893–902. https://doi.org/10.1002/jat.2911.

(7) Lee, M. H.; Chung, S. W.; Kang, B. Y.; Park, J.; Lee, C. H.; Hwang, S. Y.; Kim, T. S. Enhanced Interleukin-4 Production in CD4+ T Cells and Elevated Immunoglobulin E Levels in Antigen-Primed Mice by Bisphenol A and Nonylphenol, Endocrine Disruptors: Involvement of Nuclear Factor-AT and CA2+. *Immunology* **2003**, *109* (1), 76–86. https://doi.org/10.1046/j.1365-2567.2003.01631.x.

(8) Covaci, A.; Gerecke, A. C.; Law, R. J.; Voorspoels, S.; Kohler, M.; Heeb, N. V.; Leslie, H.; Allchin, C. R.; De Boer, J. Hexabromocyclododecanes (HBCDs) in the Environment and Humans: A Review. *Environ. Sci. Technol.* **2006**, *40* (12), 3679–3688. https://doi.org/10.1021/es0602492.

(9) Lam, J. C. W.; Lau, R. K. F.; Murphy, M. B.; Lam, P. K. S. Temporal Trends of Hexabromocyclododecanes (HBCDs) and Polybrominated Diphenyl Ethers (PBDEs) and Detection of Two Novel Flame Retardants in Marine Mammals from Hong Kong, South China. *Environ. Sci. Technol.* **2009**, *43* (18), 6944–6949. https://doi.org/10.1021/es901408t.

(10) Stapleton, H. M.; Dodder, N. G.; Kucklick, J. R.; Reddy, C. M.; Schantz, M. M.; Becker, P. R.; Gulland, F.; Porter, B. J.; Wise, S. A. Determination of HBCD, PBDEs and MeO-BDEs in California Sea Lions (*Zalophus californianus*) Stranded between 1993 and 2003. *Mar. Pollut. Bull.* **2006**, *52* (5), 522–531. https://doi.org/10.1016/j.marpolbul.2005.09.045.

(11) Birnbaum, L. S.; Staskal, D. F. Brominated Flame Retardants: Cause for Concern? *Environ. Health Perspect.* **2004**, *112* (1), 9–17. https://doi.org/10.1289/ehp.6559.

(12) Palace, V. P.; Pleskach, K.; Halldorson, T.; Danell, R.; Wautier, K.; Evans, B.; Alaee, M.; Marvin, C.; Tomy, G. T. Biotransformation Enzymes and Thyroid Axis Disruption in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Hexabromocyclododecane

Diastereoisomers. *Environ. Sci. Technol.* **2008**, *42* (6), 1967–1972. https://doi.org/10.1021/es702565h.

(13) Marvin, C. H.; Tomy, G. T.; Armitage, J. M.; Arnot, J. A.; McCarty, L.; Covaci, A.; Palace, V. Hexabromocyclododecane: Current Understanding of Chemistry, Environmental Fate and Toxicology and Implications for Global Management. *Environ. Sci. Technol.* **2011**, *45* (20), 8613–8623. https://doi.org/10.1021/es201548c.

(14) Singer, H.; Müller, S.; Tixier, C.; Pillonel, L. Triclosan: Occurrence and Fate of a Widely Used Biocide in the Aquatic Environment: Field Measurements in Wastewater Treatment Plants, Surface Waters, and Lake Sediments. *Environ. Sci. Technol.* **2002**, *36* (23), 4998–5004. https://doi.org/10.1021/es025750i.

(15) Boyd, G. R.; Reemtsma, H.; Grimm, D. A.; Mitra, S. Pharmaceuticals and Personal Care Products (PPCPs) in Surface and Treated Waters of Louisiana, USA and Ontario, Canada. *Sci. Total Environ.* **2003**, *311* (1–3), 135–149. https://doi.org/10.1016/S0048-9697(03)00138-4.

(16) Fair, P. A.; Lee, H. B.; Adams, J.; Darling, C.; Pacepavicius, G.; Alaee, M.; Bossart, G. D.; Henry, N.; Muir, D. Occurrence of Triclosan in Plasma of Wild Atlantic Bottlenose Dolphins (*Tursiops truncatus*) and in Their Environment. *Environ. Pollut.* **2009**, *157* (8–9), 2248–2254. https://doi.org/10.1016/j.envpol.2009.04.002.

(17) Dann, A. B.; Hontela, A. Triclosan: Environmental Exposure, Toxicity and Mechanisms of Action. *J. Appl. Toxicol.* **2011**, *31* (4), 285–311. https://doi.org/10.1002/jat.1660.

(18) Aranami, K.; Readman, J. W. Photolytic Degradation of Triclosan in Freshwater and Seawater. *Chemosphere* **2007**, *66* (6), 1052–1056. https://doi.org/10.1016/j.chemosphere.2006.07.010.

(19) Lindström, A.; Buerge, I. J.; Poiger, T.; Bergqvist, P. A.; Müller, M. D.; Buser, H. R. Occurrence and Environmental Behavior of the Bactericide Triclosan and Its Methyl Derivative in Surface Waters and in Wastewater. *Environ. Sci. Technol.* **2002**, *36* (11), 2322–2329. https://doi.org/10.1021/es0114254.

(20) Fu, J.; Tan, Y. X. R.; Gong, Z.; Bae, S. The Toxic Effect of Triclosan and Methyl-Triclosan on Biological Pathways Revealed by Metabolomics and Gene Expression in Zebrafish Embryos. *Ecotoxicol. Environ. Saf.* **2020**, *189* (1), 110039. https://doi.org/10.1016/j.ecoenv.2019.110039.

(21) Balmer, M. E.; Poiger, T.; Droz, C.; Romanin, K.; Bergqvist, P. A.; Müller, M. D.; Buser, H. R. Occurrence of Methyl Triclosan, a Transformation Product of the Bactericide Triclosan, in Fish from Various Lakes in Switzerland. *Environ. Sci. Technol*. **2004**, *38* (2), 390–395. https://doi.org/10.1021/es030068p.

(22) Dhillon, G. S.; Kaur, S.; Pulicharla, R.; Brar, S. K.; Cledón, M.; Verma, M.; Surampalli, R. Y. Triclosan: Current Status, Occurrence, Environmental Risks and Bioaccumulation Potential. *Int. J. Environ. Res. Public Health* **2015**, *12* (5), 5657–5684. https://doi.org/10.3390/ijerph120505657.

(23) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; Voogt, P. De; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integr. Environ. Assess. Manag.* **2011**, *7* (4), 513–541. https://doi.org/10.1002/ieam.258.

(24) Sinclair, G. M.; Long, S. M.; Jones, O. A. H. What Are the Effects of PFAS Exposure at Environmentally Relevant Concentrations? *Chemosphere* **2020**, *258*, 127340. https://doi.org/10.1016/j.chemosphere.2020.127340.

(25) Spaan, K. M.; Van Noordenburg, C.; Plassmann, M. M.; Schultes, L.; Shaw, S.; Berger, M.; Heide-Jørgensen, M. P.; Rosing-Asvid, A.; Granquist, S. M.; Dietz, R.; Sonne, C.; Rigét, F.; Roos, A.; Benskin, J. P. Fluorine Mass Balance and Suspect Screening in Marine Mammals from the Northern Hemisphere. *Environ. Sci. Technol.* **2020**, *54* (7), 4046–4058. https://doi.org/10.1021/acs.est.9b06773.

(26) Jones, P. D.; Hu, W.; De Coen, W.; Newsted, J. L.; Giesy, J. P. Binding of Perfluorinated Fatty Acids to Serum Proteins. *Environ. Toxicol. Chem.* **2003**, *22* (11), 2639–2649. https://doi.org/10.1897/02-553.

(27) Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J. F.; Hansen, K. J.; Jones, P. D.; Helle, E.; Nyman, M.; Giesy, J. P. Accumulation of Perfluorooctane Sulfonate in Marine Mammals. *Environ. Sci. Technol.* **2001**, *35* (8), 1593–1598. https://doi.org/10.1021/es001873w.

(28) Hu, W.; Jones, P. D.; Upham, B. L.; Trosko, J. E.; Lau, C.; Giesy, J. P. Inhibition of Gap Junctional Intercellular Communication by Perfluorinated Compounds in Rat Liver and Dolphin Kidney Epithelial Cell Lines in Vitro and Sprague-Dawley Rats in Vivo. *Toxicol. Sci.* **2002**, *68* (2), 429–436. https://doi.org/10.1093/toxsci/68.2.429.

(29) Hoff, P. T.; Van de Vijver, K.; Van Dongen, W.; Esmans, E. L.; Blust, R.; De Coen, W. M. Perfluorooctane Sulfonic Acid in Bib (*Trisopterus luscus*) and Plaice (*Pleuronectes platessa*) from the Western Scheldt and the Belgian North Sea: Distribution and Biochemical Effects. *Environ. Toxicol. Chem.* **2003**, *22* (3), 608–614. https://doi.org/10.1897/1551-5028(2003)022<0608:PSAIBT>2.0.CO;2.

(30) Raverty, S.; Gaydos, J.; Leger, J. *Killer Whale (Orcinus orca) Necropsy and Disease Testing Protocol*; 2014.

(31) Raverty, S.; Duignan, P.; Jepson, P.; Morell, M. Marine Mammal Gross Necropsy. *CRC Handb. Mar. Mammal Med.* **2018**, 249–268.

(32) Raverty, S.; Leger, J. S.; Noren, D. P.; Huntington, K. B.; Rotstein, D. S.; Gulland, F. M. D.; Ford, J. K. B.; Bradley Hanson, M.; Lambourn, D. M.; Huggins, J.; Delaney, M. A.; Spaven, L.; Rowles, T.; Barre, L.; Cottrell, P.; Ellis, G.; Goldstein, T.; Terio, K.; Duffield, D.; Rice, J.; Gaydos, J. K. Pathology Findings and Correlation with Body Condition Index in Stranded Killer Whales (*Orcinus orca*) in the Northeastern Pacific and Hawaii from 2004 to 2013. *PLoS One* **2020**, *15* (12 December), 1–31. https://doi.org/10.1371/journal.pone.0242505.

(33) PubChem. https://pubchem.ncbi.nlm.nih.gov/.

(34) Salgueiro-González, N.; Turnes-Carou, I.; Muniategui-Lorenzo, S.; López-Mahía, P.; Prada-Rodríguez, D. Membrane Assisted Solvent Extraction Coupled with Liquid Chromatography Tandem Mass Spectrometry Applied to the Analysis of Alkylphenols in Water Samples. *J. Chromatogr. A* **2013**, *1281*, 46–53. https://doi.org/10.1016/j.chroma.2013.01.069.

(35) Ahel, M.; Giger, W. Partitioning of Alkylphenols and Alkylphenol Polyethoxylates between Water and Organic Solvents. *Chemosphere* **1993**, *26* (8), 1471–1478. https://doi.org/10.1016/0045-6535(93)90214-P.

(36) Rüdel, H.; Böhmer, W.; Müller, M.; Fliedner, A.; Ricking, M.; Teubner, D.; Schröter-Kermani, C. Retrospective Study of Triclosan and Methyl-Triclosan Residues in Fish and Suspended Particulate Matter: Results from the German Environmental Specimen Bank. *Chemosphere* **2013**, *91* (11), 1517–1524. https://doi.org/10.1016/j.chemosphere.2012.12.030.

(37) Hayward, S. J.; Lei, Y. D.; Wania, F. Comparative Evaluation of Three High-Performance Liquid Chromatography-Based Kow Estimation Methods for Highly Hydrophobic Organic Compounds: Polybrominated Diphenyl Ethers and Hexabromocyclododecane. *Environ. Toxicol. Chem.* **2006**, *25* (8), 2018–2027. https://doi.org/10.1897/05-675R.1.

(38) Allendorf, F.; Berger, U.; Goss, K. U.; Ulrich, N. Partition Coefficients of Four Perfluoroalkyl Acid Alternatives between Bovine Serum Albumin (BSA) and Water in Comparison to Ten Classical Perfluoroalkyl Acids. *Environ. Sci. Process. Impacts* **2019**, *21* (11), 1852–1863. https://doi.org/10.1039/c9em00290a.

(39) Wang, Z.; MacLeod, M.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Using COSMOtherm to Predict Physicochemical Properties of Poly- and Perfluorinated Alkyl Substances (PFASs). *Environ. Chem.* **2011**, *8* (4), 389–398. https://doi.org/10.1071/EN10143.

(40) Gebbink, W. A.; Bossi, R.; Rigét, F. F.; Rosing-Asvid, A.; Sonne, C.; Dietz, R. Observation of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in Greenland Marine Mammals. *Chemosphere* **2016**, *144*, 2384–2391. https://doi.org/10.1016/j.chemosphere.2015.10.116.

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Marine toxic contaminants

The Washington Department of Fish and

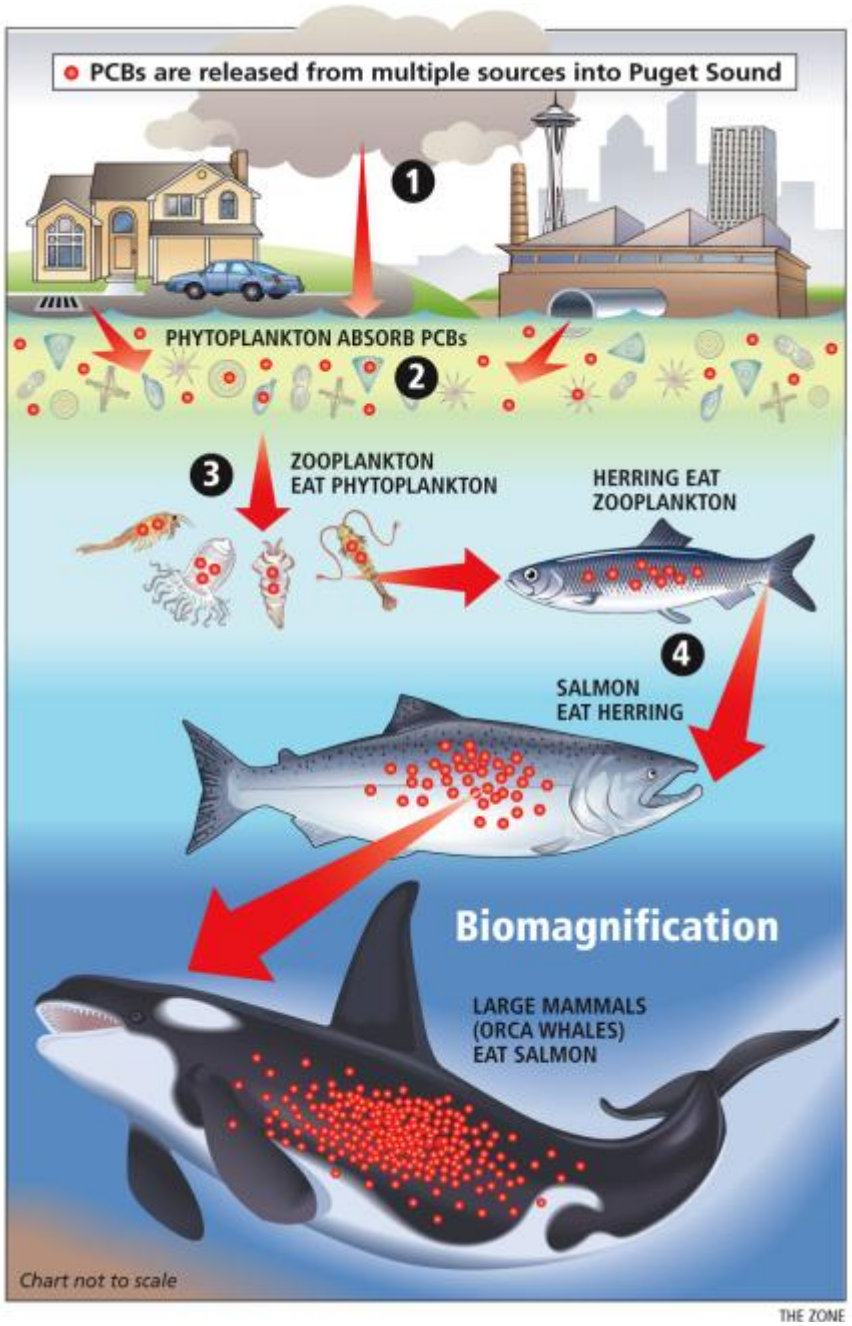

Photo by WDFW

Wildlife's Toxics Biological Observation System (TBiOS) team monitors the geographic extent and magnitude of toxic contaminants in marine and

invasive species (/species-habitats/invasive)

Wildlife diseases (/species-habitats/diseases)

Amphibians and reptiles of Washington (/species-habitats/amphibians-reptiles)

Marine toxic contaminants (/species-habitats/science/marine-toxics)

Wildlife viewing (/species-habitats/wildlife-viewing)

salmonid species in Puget Sound and Washington's Pacific coast. We evaluate and track complex patterns of pollution in these ecosystems using a number of indicator species within the food web, representing a wide range of habitat and movement patterns. Results from these studies help guide efforts to protect fish and shellfish health, ensure seafood safety, and promote ecosystem recovery.

Toxics Biological Observation System (TBiOS) (/species-habitats/science/marine-toxics/tbios)

WDFW'S TBiOS team monitors the geographic extent and magnitude of toxic contaminants in fish and shellfish living in Puget Sound and Washington's coast.

Study design (/species-habitats/science/marine-toxics/study-design)

TBiOS monitors spatial and temporal trends of contaminant exposure in Puget Sound fish and shellfish and the effects of that exposure on the health of these resources.

Sampling locations (/species-habitats/science/marine-toxics/sampling-locations)

Locations where TBiOS collects and evaluates levels of contaminants in various fish and shellfish species.

Indicator species (/species-habitats/science/marine-toxics/species-monitored)

TBiOS monitors toxic contaminants in a number of indicator species within the food web, including Pacific herring, English sole, blue mussels, and Pacific salmon.

Contaminants monitored (/species-habitats/science/marine-toxics/contaminants-monitored)

TBiOS monitors a wide range of toxic contaminants such as PCBs, PBDEs, DDTs, PAHs, inorganic metals and chemicals of emerging concern.

Publications (/species-habitats/science/marine-toxics/publications)

TBiOS publications, reports and Quality Assurance Project Plans (QAPPs).

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Environ Toxicol Chem. 2018 Oct;37(10):2692-2698. doi: 10.1002/etc.4240. Epub 2018 Sep 5.

Insecticide-induced changes in amphibian brains: How sublethal concentrations of chlorpyrifos directly affect neurodevelopment

Sara J McClelland [1], Randall J Bendis [2], Rick A Relyea [3], Sarah K Woodley [1]

Affiliations

PMID: 30187530 DOI: 10.1002/etc.4240

Abstract

Widespread use of pesticides often contaminates natural habitats, exposing nontarget organisms to pesticides that were designed to control pest populations. Even low levels of pesticides can affect aquatic communities both directly and indirectly. Previous work has shown that trace amounts of the pesticide chlorpyrifos altered tadpole morphology and neurodevelopment in artificial ponds (mesocosms). To determine whether effects resulted from direct chlorpyrifos exposure or from disruption of the food web due to a pesticide-induced decline in zooplankton, we examined the impacts of chlorpyrifos on amphibian development in the presence of chlorpyrifos-resistant zooplankton, a key component of the aquatic trophic community. Northern leopard frog (Lithobates pipiens) tadpoles were reared through metamorphosis in mesocosms containing either 0 or 1 µg/L chlorpyrifos and either chlorpyrifos-resistant or chlorpyrifos-sensitive Daphnia pulex zooplankton. Developmental exposure to chlorpyrifos resulted in metamorphs with a relatively wider optic tectum, medulla, and diencephalon compared with controls, and this result was found regardless of the zooplankton population within the mesocosm. Thus, chlorpyrifos directly impacted brain development, independent of the effects on the trophic community. With respect to body shape, chlorpyrifos had no effect on body shape of metamorphs reared in mesocosms with chlorpyrifos-sensitive zooplankton, but body shape was sensitive to zooplankton population in the absence of chlorpyrifos. To conclude, low, ecologically relevant doses of organophosphorous pesticides can directly impact neurodevelopment in a vertebrate model. Environ Toxicol Chem 2018;37:2692-2698. © 2018 SETAC.

Keywords: Acetylcholinesterase inhibitors; Gape width; Insecticide; Neurology; Organophosphate; Rana pipiens.

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Organotins in Aquatic Biota: Occurrence in Tissue and Toxicological Significance

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7 Organotins in Aquatic Biota
Occurrence in Tissue and Toxicological Significance

James P. Meador

CONTENTS

7.1 INTRODUCTION

Organotins are organometallic compounds that exhibit complex environmental chemistry and toxicity. The handbook of Chemistry and Physics (CRC 1975) lists more than 250 organotin compounds. Even though a number of these are specific compounds (e.g., triphenyltin [TPT]) that are listed as various salts (e.g., TPT chloride, sulfide, hydroxide, and bromide), there are dozens of unique compounds. Because a number of organotins will be considered here, Table 7.1 lists the compounds and their abbreviations.

The focus for this chapter is the occurrence of organotin compounds in aquatic organisms and the associated toxic responses. For aquatic organisms, we traditionally define the effective concentration for toxicity based on the ambient-exposure pathway (e.g., water, air, soil/sediment, prey); however, tissue residues reflect the bioavailable and effective target dose more accurately than the conventional "dose." The term "dose" is loosely applied; however, it most accurately defines that bioactive fraction occurring at the site of action. Differences in the inherent toxicity (potency) of compounds within and between mechanisms of toxic action (MeOAs) are more apparent in residue-based dose metrics than exposure-based dose metrics because the influence of many confounding factors can be taken into account and avoided. For example, a high percentage of the range for LC_{50} or EC_{50} values that are based on water or sediment concentrations are due to the variability in the bioavailable fraction and the uptake and elimination rate kinetics that determine bioaccumulation (Meador 2006). When tissue residues are used as the dose metric, differences in bioavailability and bioaccumulation are greatly reduced and we are left with just the potential variability in potency that may occur among species. In many cases, the range in values for a given toxicant among all species can be reduced by 4–5 orders of magnitude for ambient-exposure toxicity metrics to one order of magnitude when tissue-based toxicity metrics are considered (Meador 2006, Meador et al. 2008). The major advantage of this feature is in assessing concentrations of a given toxicant in feral organisms. Hence, we can more accurately determine the likelihood of potential toxic effects for some chemicals measured in field-collected organisms when a low variance is observed among species.

In this chapter I will present an overview for organotins, with some basic information on their environmental chemistry, occurrence, and bioaccumulation. The available data on tissue-residue toxicity for aquatic biota will be presented and discussed. Some of the toxicity information for small mammals will also be shown for comparison and to highlight similarities among diverse taxa. In the summary, section I will provide general conclusions regarding the toxicity of organotin compounds as a function of tissue concentrations.

7.2 BACKGROUND

Organotins have several applications and are primarily used as plasticizers in industrial applications and as biocides to control so-called nuisance organisms. As pesticides they are used as antifoulants, wood preservatives, molluscicides, antihelminthics, and fungicides for textiles and various water systems (Cima et al. 2003, Antizar-Ladislao 2008). Most of the pesticides are triorganotin compounds and of this group tributyltin (TBT) and TPT are the most commonly encountered environmental contaminants because of their biocidal properties and widespread use on boat hulls to prevent the accumulation of fouling organisms. TPT is also applied to some crops as a fungicide and will therefore likely leach into watersheds. Organotins are also used as heat and light stabilizers for

TABLE 7.1
Organotin Properties

Compound	Abbrev	MW	Log_{10} K_{ow}	pKa	Predicted BCF	Convert ng Sn/g to ng OT/g	Convert µg OT/g to nmol/g
Tetramethyltin	TeMT	179	−2.2	na	9×10^{-5}	1.50	5.59
Trimethyltin	TMT	164	−2.3	6.6	8×10^{-5}	1.38	6.10
Dimethyltin	DMT	148	−3.1	3.5	1×10^{-5}	1.24	6.76
Monomethyltin	MMT	135	−3.1	2.6	1×10^{-5}	1.13	7.41
Tetraethyltin	TeET	233	—	—	—	1.97	4.29
Triethyltin	TET	205	−1.8	6.8	2×10^{-4}	1.74	4.88
Diethyltin	DET	177	−1.4	3.7	6×10^{-4}	1.49	5.65
Tetrapropyltin	TePrT	291	2.0	na	1.50	2.45	3.44
Tripropyltin	TPrT	248	0.9	6.3	0.12	2.08	4.03
Dipropyltin	DPrT	205	na	na	—	1.72	4.88
Monopropyltin	MPrT	161	na	na	—	1.35	6.21
Tetrabutyltin	TeBT	347	3.9	na	119	2.92	2.88
Tributyltin	TBT	283	4.4	6.5	377	2.44	3.45
Dibutyltin	DBT	233	1.3	3.8	0.30	1.96	4.29
Monobutyltin	MBT	177	0.4	2.0	0.04	1.49	5.65
Tetraphenyltin	TePT	427	4.4	na	377	3.59	2.34
Triphenyltin	TPT	350	3.6	5.2	60	2.94	2.86
Diphenyltin	DPT	273	1.9	2.7	1.2	2.29	3.66
Monophenyltin	MPT	197	1.2	na	0.24	1.66	5.08
Tri-*n*-hexyltin	TnHT	375	3.7	na	75	3.15	2.67
Tri-*c*-hexyltin	TcHT	375	4.1	na	189	3.15	2.67
Azocyclotin	ACT	436	5.4	na	3598	3.66	2.29
Dihexyltin	DHT	298	na	na	—	2.50	3.36
Trioctyltin	TOT	458	na	na	—	3.85	2.18
Dioctyltin	DOT	355	5.8	na	9910	2.98	2.82
Monooctyltin	MOT	252	2.1	na	2.1	2.12	3.97
Inorganic tin	Sn	119	na	na	—	—	—

Many of the log_{10} K_{ow} values were calculated or determined at unspecified pH. Data for tributyltin, triphenyltin, and trihexyltin from Fent (1996), Meador (2000), Arnold et al. (1997), and Tas (1993) were determined at circumneutral pH. Most other data from Wong et al. (1982) and Vighi and Calamari (1985). Convert to ng OT/g is the value to multiply ng Sn/g for the result. Covert to nmol/g is the value to multiply µg OT/g for the result (dividing nmol/g by this factor equals µg OT/g). Predicted BCF based on K_{ow} QSAR for ionization-corrected substituted phenols, BCF = $K_{ow} \times 0.015$ (McCarty 1986). MW is the molecular weight, K_{ow} is the octanol–water partition coefficient, OT is organotin, and na is not available. Note, most organotins are reported as the ionic concentration (i.e., without the anion such as Cl or OH) except for the tetra substituted or neutral forms.

polyvinyl chloride (PVC) plastics and as catalysts for various chemical reactions and account for approximately 70% of the total production (mostly dibutyltins [DBTs]) (Cima et al. 2003). DBT is used as a biocide to treat chickens for tapeworm, and it is also a metabolite of TBT, thus commonly found in tissue after TBT exposure. Also, because this compound is used in PVC production, it will leach into aquatic systems from pipes made of this plastic. Most of the organotin research has been conducted on TBT, although there are several other organotins (e.g., TPT, fenbutatin, azocyclotin, and hexamethylditin) that are widely used, mostly as agricultural pesticides that can end up in aquatic systems.

7.2.1 Legislation

Organotins as antifoulants were introduced early in the 1970s and widely used throughout the 1980s. Late in the 1980s, several countries around the world and states within the United States enacted restrictions on the use of organotins as antifoulants. In 1988, the U.S. government enacted the Organotin Antifouling Paint Control Act of 1988 (OAPAC; U.S. Congress 1988), which restricted the use of tin-based antifoulants on small vessels based on the size of the vessel (<25 m in length) and release rate from the paint surface (4 μg TBT/cm^2/day).

An International Convention (treaty) was adopted in 2001 by the International Maritime Organization to prohibit the use of TBT by 2008 (IMO 2001). This treaty came into force in September of 2008 and it requires signatories to prohibit the use of harmful antifoulants (organotins) on ships flagged in their country and to deny entry into their ports for any foreign ship using such antifoulants.

7.2.2 Reporting Concentrations

Many studies report organotin concentrations as ng Sn/g. This is mostly a result of the standard analytical method (flame photometric detection) that quantifies tin concentrations using a tin-selective detector. Reporting organotins as ng Sn/g is misleading because the whole molecule is responsible for the toxic response as a function of its interaction with a receptor. The organotin is the active molecule causing toxicity, not elemental tin (Sn). There are many metal-containing compounds that are not reported in these terms. For example, methyl mercury is not reported as ng Hg/g nor is hemoglobin reported as mg Fe/L. When an organotin is expressed as ng Sn/g the variability among organotins and their toxic potency is masked. In addition, it is generally not appropriate to report the concentration of the various salts or complexes (e.g., tributyltin chloride [TBTCl] or tributyltin hydroxide [TBTOH], bis(tributyltin) oxide [bis-TBTO]) mainly because the standard analytical techniques for quantitation can not distinguish between these species, and it is not known which form (salt or ion) is the toxic species of concern. Once these compounds are introduced to water or tissue, they speciate according to the pH and ionic composition of the receiving water or fluid. Even though TBTOH may be the predominant species that is bioaccumulated, TBT is also found as many different species in plasma and tissue.

In comparing specific compounds, molar concentration is more appropriate because the toxic response is more closely related to the number of molecules interacting with the receptor, not their mass per unit organism weight. Table 7.1 provides conversion factors for mass to molar concentrations and for converting ng Sn/g to ng organotin/g. Organotin concentrations in this review are reported as mass or molar concentrations [e.g., ng or nmol organotin/unit matrix (e.g., g or mL)]. Most organotins are reported as ng organotin/g, except those that are neutral species. Also, all tissue concentrations are reported in terms of wet weight (ww) (unless noted), and body weight is abbreviated as "bw." Many tissue concentrations were originally reported as dry weights, which were converted to wet weights for this review by multiplying the value by 0.2, a standard conversion factor for fish and invertebrate tissue.

The lethal and effective residue designations (LR_p and ER_p) are used in this review to denote the residue (tissue concentration) associated with a response (Meador 1997). The term "effective residue" or "effective concentration" is normally used to characterize a sublethal response and the "p" for each represents the percent or proportion responding. Similar designations are used for the lowest observable effect residue (LOER) and no observable effect residue (NOER). These metrics represent the internal acquired dose, which is generally not equivalent to the externally administered dose. These values (LR_p and ER_p) are distinguished from lethal dose (LD_p) or effective dose (ED_p) values, which are usually based on the administered dose (i.e., dietary as a daily or one-time dose; μg toxicant/g bw/day or μg toxicant/g bw). The administered dose is frequently not the concentration associated with the response, which is our main interest for this review (Meador 2006).

7.3 ENVIRONMENTAL CHEMISTRY

Organotin compounds are a combination of organic moiety and elemental tin (Sn). All organotin compounds contain a metal–carbon bond, and in many cases the organic moiety is an alkyl group or simple ring structure. The most common alkyl moieties are the methyl, ethyl, propyl, butyl, hexyl, and octyl groups and these may occur in series (e.g., methyl, dimethyl, and trimethyl). Tin is also found in coordination with other groups (e.g., phenyl, cyclohexyl, and others) and these can also occur in series (tri-, di-, and monosubstituted). The presence of various organic moieties greatly enhances the compound's hydrophobicity, which increases its bioavailability and toxicity. A list of various organotin compounds and their key physical-chemistry properties can be found in Table 7.1. Some organotin compounds found in the environment are not listed because no data were found. As a result of the increased hydrophobicity, organotins are readily bioaccumulated by organisms and may be more persistent in tissues; however, predicting the bioaccumulated amount is complex.

The most common organotins in aquatic environments occur as triorganotins (e.g., TBT, TPT, trimethyltin [TMT], tripropyltin [TPrT], etc.), diorganotins (DBT, dimethyltin [DMT], diethyltin [DET], etc.), and monoorganotins such as monobutyltin (MBT), and monomethyltin (MMT). There are a very large number of potentially toxic organotins and many of these are found in the environment and are considered significant contaminants. Unfortunately, we know very little about the occurrence, bioaccumulation, and toxicity for most organotins. Organotin environmental chemistry is relatively complex because these compounds are often polar, ionizable, and hydrophobic.

7.3.1 Octanol–Water Partition Coefficient

A very useful chemical parameter for predicting the partitioning behavior between water, sediment, and tissue for some organic compounds is the octanol–water partition coefficient (K_{ow}). The K_{ow} is a surrogate measure of the association of organic compounds with lipid or organic carbon. In many cases the K_{ow} is used in quantitative-structure activity relationships (QSARs) to predict sediment-water or water-tissue partitioning. Even though organotins exhibit strong partitioning to lipid and organic carbon (Meador 2000, Brändli et al. 2009) these QSARs do not always predict chemical behavior. In some cases QSARs for sediment-water partitioning are fairly accurate (Meador 2000); however, those for water-tissue partitioning generally are far from predictive. The available K_{ow} values (as $\log_{10}$) for organotin compounds are listed in Table 7.1, and it is important to note the large variability among organotins. The very low (some negative) $\log_{10} K_{ow}$ values indicate that these organotins may not bioaccumulate as much as others; however, bioaccumulation of individual organotins is likely not related to K_{ow} or lipid content (Meador 2000). Surprisingly, many organotins exhibit very low K_{ow} values, which would indicate a low potential to cross biological membranes; however, some exhibit high bioaccumulation factors.

7.3.2 Hydrogen Ion Activity (pH) and the Acid-Dissociation Constant (pKa)

For ionizable organometallic compounds, hydrogen ion activity (pH) and the acid-dissociation constant (pKa) appear to be important chemical controlling factors. Many organotins are ionizable; therefore, pH can have a strong effect on partitioning. Because neutral chemical species, such as TBTOH, are generally more bioavailable for passive diffusion than the ionized form, the proportion of the total compound in solution that is in the neutral form is important for bioaccumulation assessment. Organotins are generally cations and when the pH of a solution containing an ionizable organotin is equal to the pKa, the molecules are equally apportioned between ionized and unionized forms. As the pH increases above the pKa, more of the organotin will be in the neutral form and available for uptake.

As shown by Tsuda et al. (1990) and Arnold et al. (1997) the octanol–water partition coefficient (K_{ow}) for TBT is strongly affected by pH. From a pH of 5.8 to 8.0, the K_{ow} increases from 1600 to

12,000. TPT is also affected by pH and for this same range in pH the K_{ow} increases from 1180 to 3650 (Tsuda et al. 1990). This is an important factor for assessing bioaccumulation because it is the hydrophobic portion that partitions into octanol and this is generally the bioavailable form. As seen in Table 7.1, the pKa for these compounds ranges between 2 and 7, indicating that in most aquatic systems with an alkaline pH, the neutral forms, such as hydroxide species, will predominate.

7.3.3 Carbon

Water-sediment partitioning and bioaccumulation of organotins are known to be affected by the organic carbon and black carbon content in sediment and dissolved organic carbon in the water column (Fent 1996, Meador 2000, Hoch and Schwesig 2004, Veltman et al. 2006, Brändli et al. 2009). Carbon content can affect the amount of free TBT that is available for uptake because of its predominant hydroxide form in aquatic systems that will complex with dissolved or particulate carbon.

7.4 ENVIRONMENTAL OCCURRENCE

7.4.1 Water and Sediment

TBT has been reported by several authors in the water column at concentrations commonly ranging from 1 to 200 ng/L in harbors and marinas around the world (Seligman et al. 1989, Fent 1996, Antizar-Ladislao 2008, Harino et al. 2008). In a few cases, aqueous concentrations have been extremely high (500–2000 ng/L) (Clark and Steritt 1988, Antizar-Ladislao 2008); however, observations in this range were not common.

When restrictions on the use of TBT as an antifouling paint were enacted in the late 1980s in many countries, water concentrations declined (Fent 1996); however, sediment concentrations remained relatively high (Krone et al. 1996, Antizar-Ladislao 2008). Even though water concentrations in the United States declined after OAPCA was enacted, levels were still somewhat elevated because it did not impact use on most commercial ships. Due to the large number of small vessels, these restrictions were generally effective causing substantial reductions in aqueous concentrations (Huggett et al. 1992), but less so for sediment. This has also been observed in coastal waters around Japan where the use of TBT and TPT were restricted in 1989 (Harino et al. 2008). The Harino et al. (2008) review on TBT and TPT occurrence in water, sediment, and tissue found large decreases in concentrations shortly after the restrictions were enacted, but only minor reductions were observed after reaching lower levels. These reduced concentrations are still relatively high compared to toxic levels, with water concentrations averaging 11 ng/L (range <1–83 ng/L) and highly variable sediment values (<1–650 ng/g).

TBT appears to be very persistent in sediment and concentrations in the hundreds of parts per billion (ppb) to low parts per million (ppm) range can still be found in harbors and marinas in various countries (de Mora et al. 1989, Fent 1996, Antizar-Ladislao 2008). Although TBT may be quickly degraded to DBT and MBT in the water column (Seligman et al. 1988), degradation appears to be much slower once it is associated with sediment. One study determined half-lives of TBT in sediment ranging from 1 to 2 years in surficial aerobic sediment (Dowson et al. 1996); however, they reported essentially no degradation in anaerobic sediment. These estimates are supported by other studies that observed long half-lives for TBT in sediment (Hwang et al. 1999, Takahashi et al. 1999). As a result of these long half-lives, sediment-associated TBT will likely continue to be a source and lead to elevated water and tissue concentrations.

Fent (1996) provides an excellent review of the environmental concentrations that were found from the early 1980s through the mid-1990s. It is striking to note the extent and frequency of observed sediment concentrations in the low ppm range (i.e., 1–10 μg/g) and water concentrations in the high parts per trillion (pptr) range (0.1–1.0 ng/mL). These elevated concentrations certainly resulted in severe biological effects in many ecosystems considering that the EPA chronic water

quality criteria (U.S. EPA 2003) is set to 0.07 ng/mL in freshwater and 0.007 ng/mL in marine ecosystems.

7.4.2 Tissue

7.4.2.1 Butyltins

Most of the available tissue concentration data are for TBT (and its metabolites DBT and MBT) because it is a commonly applied pesticide and is extremely toxic. There are several excellent reviews that provide tables of butyltin concentrations in aquatic species (Tanabe 1999, Maguire 2000, Birchenough et al. 2002, Shim et al. 2005). A recent review article provided an overview of measured tissue concentrations in a variety of fish and invertebrates from American, Asian, and European harbors and marinas (Antizar-Ladislao 2008). As expected the range in tissue levels is very broad, although many of the species exhibit relatively high concentrations (hundreds of ng/g ww) for all butyltin compounds. Shim et al. (2005) presented soft-tissue concentrations of butyltins and TPT in five bivalve species collected worldwide. Most of those samples span from the late 1980s through the 1990s and show very high concentrations for most samples. In this chapter (Table 7.2), we have listed some additional recent tissue concentration data for butyltins. These recent values also indicate very high values for some locations and species.

7.4.2.2 Phenyltins

Phenyltins are also applied as an antifoulant and consequently are commonly found in the tissues of field-collected aquatic organisms. In many cases, TPT is found at elevated and similar concentrations to that observed for TBT (Tolosa et al. 1992, Shim et al. 2005) with concentrations occurring in the range of hundreds of ng/g ww (Table 7.2). A comprehensive review of TPT tissue concentrations in the muscle of wild fish from around the world (n = 20 species) found high mean concentrations with many in the 200–600 ng/g ww range (Zhang et al. 2008, Table S3 in supporting information). The review by Shim et al. (2005) also indicates high TPT concentrations (up to 5930 ng/g ww) for many of the bivalve samples from several locations (Korea, Japan, Mediterranean, and The Netherlands). High levels were also reported by Harino et al. (2008) for TPT in mussel tissue (up to 3400 ng/g) in their review of data from Japanese coastal waters.

7.4.2.3 Other Organotins

Essentially all of the other tissue concentration data for organotins consists of values for the TBT and TPT metabolites (DBT, MBT, diphenyltin [DPT], and monophenyltin [MPT]). After extensive searching, very few studies were found that reported tissue concentrations for other organotins in field-collected aquatic animals. One study examined various seafood species for tetramethyltin (TeMT), tetraethyltin (TeET), and TMT (Forsyth and Clerous 1991). No concentrations above the method detection limit (MDL) were observed for TeMT (MDL = 1.2 ng/g) and TeET (MDL = 1.4 ng/g). TMT was found in cockles (1.0 ng/g) and turbot (3.9 ng/g).

7.4.3 Observations

One monitoring study found no decline in TBT over time in mussels from the North Sea even though this antifoulant was banned on small boats there in 1991 (Rüdel et al. 2003). The authors concluded that the absence of decline for tissue residues was likely due to inputs from large vessels that were still using TBT. The recently enforced IMO ban may produce reductions in water concentrations, however, due to the extensive half life of TBT (and likely other organotins) in sediment; these compounds will likely continue to be a concern for many years.

The vast majority of reported tissue concentrations for organotin compounds are for marine species; however, there are studies that examined these compounds in freshwater ecosystems. One

TABLE 7.2
Recent Data on Occurrence of Butyltins and Phenyltins in Aquatic Organisms

Organotin	Spp.	Type	Tissue	D/W	ng ion/g	*n*	Site	Reference
					Butyltins			
TBT, DBT, MBT	O.o.	Marine mammal	Liver	W	19 (8), 298 (192), 77 (51)	5	Rausu, Hokkaido, Japan	Harino et al. (2008)
TBT	C.g.	Oyster	Soft tissue	D	263–10,562	337	Luerman Estuary, Taiwan	Tang and Wang (2008)
TBT	P.v.	Mussel	Soft tissue	D	209–14,000	242	Luerman Estuary, Taiwan	Tang and Wang (2008)
DBT	10 species	Fish	Whole fish	W	<dl–276	27	Several U.S. fw sites	Jones-Lepp et al. (2004)
TBT	Unspec	Mussel	Whole	W	4–381	6 sites	Coastal Japan	Harino et al. (2008)
TBT	15 species	Fish and inverts	Unknown	D	2–240	2 sites	Coastal Japan—deep water	Kono et al. (2008)
TBT, DBT	P.p.	Porpoise	Liver	W	67–266, 88–743	12 sites	Baltic	Ciesielski et al. (2004)
TBT, DBT	11 species	Marine mammal	Liver	W	20–820, 32–2900	10 sites	Worldwide	Kajiwara et al. (2006)
					Phenyltins			
TPT	C.g.	Oyster	Soft	D	882 (498)	13	South Korea	Shim et al. (2005)
TPT	M.e.	Mussel	Soft	D	1093 (1071)	5	South Korea	Shim et al. (2005)
TPT	A.p.	Starfish	Soft	D	976 (664)	26	South Korea	Shim et al. (2005)
TPT	15 species	Fish and inverts	Unknown	D	5–460	2 sites	Coastal Japan—deep water	Kono et al. (2008)
TPT	Various*	Fish and inverts	Fish muscle soft tissue inverts	W	1.2–35	48	Bohai Bay, China	Hu et al. (2006)
TPT	11 species	Fish	Muscle	W	25–130	60	Osaka, Japan	Harino et al. (2000)
TPT	10 species	Fish	Whole fish	W	<dl–500	27	Several U.S. fw sites	Jones-Lepp et al. (2004)
TPT, DPT	6 species	Fish	Muscle	W	22–1535, 2–180	32^	Lake system Westeinder, The Netherlands	Stäb et al. (1996)
TPT	Various	Inverts	Whole	W	20–543	22^	Lake system Westeinder, The Netherlands	Stäb et al. (1996)
DPT	Various	Inverts	Whole	W	<dl–736	22^	Lake system Westeinder, The Netherlands	Stäb et al. (1996)
TPT	Unspecified	Mussels	Whole	W	<dl–3400	6 sites	Coastal Japan	Harino et al. (2008)
TPT	15 species	Fish and inverts	Unknown	D	5–460	2 sites	Coastal Japan	Kono et al. (2008)
TPT, DPT, MPT	O.o.	Marine mammal	Liver, lung, blubber, muscle	W	<1–14, <1–17, <1–72	5	Rausu, Hokkaido, Japan	Harino et al. (2008)

Values are mean (standard deviation) for various tissues (tiss). C.g. = *Crassostrea gigas*, A.p. = *Asteria pectinifera*, M.e. = *Mytilus edulis*, P.v. = *Perna viridis*, O.o. = *Orcinus orca*, P.p. = *Phocoena phocoena*. Various* are samples from a number of species including phyto- and zooplankton, benthic invertebrates (inverts), and fish. *n* is the number of individuals for each site, except where number of sites with variable sample sizes indicated. D/W shows dry (D) or wet (W) weight, fw is freshwater, and dl is the detection limit. ^ denotes composite samples. Organotin (OT) abbreviations in Table 7.1.

recent study conducted a survey of DBT and TPT in whole fish from freshwater sites across the United States, which reported values from the detection limit (<1 ng/g) to 276 ng/g for DBT and <1.8–499 ng/g for TPT (Jones-Lepp et al. 2004). Some older studies reported high concentrations of TPT in freshwater organisms. These include bivalves collected in Swiss lakes (Fent 1996) and numerous invertebrates and fish from Dutch lakes (Stäb et al. 1996) (Table 7.2).

Even far offshore in deep (marine) water (100–400 m), elevated concentrations of TBT and TPT have been documented (Kono et al. 2008). These authors found several marine species with concentrations of these two organotins in the range of 2–20 ng/g ww with one value as high as 90 ng/g ww. These authors also reported water concentrations in the range of 0.3–0.8 ng/L for TBT and sediment concentrations up to 16 ng/g dry wt. for this compound and 12 ng/g dry wt. for TPT.

Marine mammals also appear to accumulate relatively high concentrations of organotins. Several recent studies and reviews demonstrate that numerous marine mammal species exhibit high levels in various tissues, including liver, blubber, and muscle. Tanabe (1999) found concentrations of TBT at high concentrations (35–2200 ng/g ww) in several different tissues of finless porpoise (*Neophocaena phocaenoides*) from waters around Japan, with similar high concentrations for DBT and MBT. A review article by Kajiwara et al. (2006) presents data for 11 marine mammals species from various locations (Japan, Great Britain, Mediterranean, United States, Indo-Pacific, and India) showing high concentrations of TBT in liver (mean values 20–820 ng/g ww, maximum = 1200 ng/g). A number of studies examined organotins in killer whales (*Orcinus orca*). Harino et al. (2008) found TBT concentrations in the range of 6–25 ng/g ww and far higher levels of DBT (16–556 ng/g) and MBT (16–152 ng/g) in the liver of this species (Table 7.2). They also report low levels of TPT (<1–58 ng/g) in blubber and liver, which was also noted by Kajiwara et al. (2006) who reported no detectable concentrations of TPT or DPT in killer whales.

7.5 ORGANOTIN BIOACCUMULATION

Assessing bioaccumulation of organotins is complex. Standard QSAR models used for organic compounds are poor predictors of accumulation for these compounds and most organotins do not behave as metals. In general, it appears the pattern of bioaccumulation among organotins is somewhat correlated to K_{ow}; however, using this parameter to predict bioaccumulation for an individual compound across species is not supportable. For example, an analysis of TBT bioaccumulation shows that it does not obey organic-compound QSAR predictions for bioaccumulation or toxicity. The predicted wet-weight bioconcentration factor (BCF) for TBT in species that do not metabolize this compound is approximately 377. This value was determined with the QSAR for ionization-corrected substituted chlorophenols (Saarikoski and Viluksela 1982; also see McCarty 1986). These compounds are known to be uncouplers of oxidative phosphorylation, as are organotins. As shown in Meador (2006), all observed TBT BCFs exceed this predicted value. For those species that exhibit weak biotransformation of this compound, the BCFs are approximately 30–250 fold higher than those predicted using the QSAR.

7.5.1 Concepts

Some of the triorganotins exhibit relatively high K_{ow} values ($\log_{10}$ values of 3–4); however, TMT, triethyltin (TET), and TPrT are all very low (Table 7.1). In general, there is a very strong association between the K_{ow} value and the number of carbons in the substituted groups among triorganotins ($r^2 = 0.80$), and based on this relationship the compounds with the highest K_{ow} value and number of carbons would be expected to exhibit the highest BCFs or BAFs. As shown later, this is not the case for some of the organotins, such as TPrT. Unfortunately, we have very few data for other organotins.

Based on the K_{ow} at pH 8.0 from Tsuda et al. (1990) the predicted BCF for TPT at steady state using the same QSAR formula derived for substituted phenols is 60. The observed steady-state BCF for carp (*Cyprinus carpio*) exposed to TPT was determined to be 600, which is 10 times higher.

Because fish are known to extensively metabolize TBT (Lee 1985), the QSAR predicted BCF for this species should be far higher than the observed value. Another study reported the TPT BCFs for two fish species (*Pagrus major* and *Rudarius ercodes*) after 56 days of exposure to be similar at 3200 and 4100, respectively, 53–68 times the predicted value. In general terms, several studies have demonstrated that BCFs for TBT are substantially higher than those for TPT (Tsuda et al. 1988, 1991, Fent 1996), which may be a result of the speciation profile that is controlled by pH, K_{ow}, or some physiologic aspect of bioaccumulation. When tissue-residue toxicity is considered, these differences become less significant because of the increased importance of toxic potency.

Many aquatic invertebrate species are known to have minimal metabolic capacity for organotins (Fent 1996), and they exhibit BCF values that are far higher than those observed for fish. As seen in Meador (2006) the BCFs for TBT in several species are very high ranging from 2000 to 95,000. Similarly, high BCF values have also been observed for invertebrates and other organotins. One study exposed marine snails (*Nucellus lapillus*) to aqueous concentrations of TPrT and reported a BCF value of approximately 15,000 after 30 days exposure (Bryan et al. 1988). Based on the bioaccumulation QSAR for this compound used earlier, the expected steady-state BCF for a species that does not metabolize TPrT is predicted to be 0.12, which is 1.3×10^5 times lower than the observed value for *N. lapillus*. No bioaccumulation is expected for TMT ($\log_{10} K_{ow}$ of −2.3); however, one study reported a BCF value of 75 for the brine shrimp (*Artemia franciscana*) (Hadjispyrou et al. 2001). The same study reported a BCF of 50 for DMT, which exhibits a $\log_{10} K_{ow}$ of −3.1 (essentially 0).

From these data we can conclude that the commonly used bioaccumulation QSAR equations can not be used to predict tissue concentrations for organotins for fish or invertebrates. Therefore, measured toxicokinetic values are the only reliable method for such predictions. This is an important point because a number of guidelines and statutes require chemical screening based on K_{ow} when assessing the potential for chemicals to bioaccumulate and cause harm. As seen earlier, these assumptions on bioaccumulation QSARs are not valid for organotins and possibly other poorly studied contaminants.

Several environmental factors are likely important for determining bioaccumulation for organotins including pH, temperature, redox state, salinity, and organic carbon content in sediment and water. Most of these factors will affect the amount of the bioavailable compound or the rate of uptake. Also, because of the large disparities between actual and predicted bioaccumulation, there must be biological factors (e.g., interspecific differences in rates of uptake, elimination processes, membrane permeability, transport mechanisms, etc.) that are prominent controlling factors for bioaccumulation. When we consider tissue-residue toxicity metrics, these factors are all far less germane because bioavailability and toxicokinetics are accounted for when toxicity is expressed as a tissue concentration (e.g., LR_p or ER_p).

7.5.2 Bioaccumulation Kinetics

A more accurate way to predict bioaccumulation is with uptake and elimination rate constants. As a simple example, the rate of uptake (uptake clearance; k_1) divided by the rate of elimination (k_2) equals the BCF at steady state.

7.5.2.1 Uptake

As mentioned earlier, pH has a large influence on hydrophobic partitioning because it determines the profile of the various organotin species. As pH increases, TBTOH becomes more abundant and the ionic form (TBT^+) decreases. A few studies have demonstrated that the rate of uptake increases with increasing pH for TBT (Fent 1996) and TPT (Tsuda et al. 1990), which is likely a result of the reduction in the ionic species and an increase in neutral species (e.g., TBTOH). Therefore pH impacts the rate of uptake only because it affects the proportions of the various organotin species in the exposure media. Because of these differences due to pH, marine organisms often exhibit higher BCF values than freshwater species for organotins because the pH of freshwater is often lower than seawater (≈8.1).

We know from several studies that rate of uptake for TBT is highly variable among species. As an example, Meador (1997) observed order of magnitude differences in TBT BCF values and toxicokinetics for two similar amphipods under identical environmental conditions. It is not known if these results are a function of ventilation rate, membrane permeability, or other physiological or morphological differences among species.

7.5.2.2 Elimination

The rate of elimination includes the processes of metabolism, passive diffusion, and excretion. Because total elimination values are calculated, we do not know what portion was metabolized and how much of the parent compound was lost through passive or active processes. Elimination for many organotins is accomplished by metabolic transformation, which occurs via the cytochrome P450 enzyme system that facilitates the degradation of a large number of xenobiotics (Fent 1996). For example, TBT is sequentially debutylated in a series of reactions with the cytochrome P450 system (TBT → DBT → MBT → Sn) (Fent 1996). All these metabolites will be measured in organisms that can metabolize TBT and are exposed to this compound for several days (Meador 1997). TPT is metabolized in a similar fashion (TPT → DPT → MPT → Sn) (Fent 1996). A few studies have compared the elimination rates of TBT and TPT in fish and found that TBT was generally more rapidly eliminated than TPT (Tsuda et al. 1988, 1992) indicating that TPT may persist longer in tissue. A low rate of elimination (k_2) for TPT was also noted by Stoner (1966) for guinea pigs.

For fish species, metabolic rates (k_m) for organotins should be substantial due to the high levels of cytochrome P450. In general, rates of biotransformation via P450 are known to be highly variable among invertebrate taxa (Livingstone 1998). Unfortunately, most studies that examine the elimination of organotins from tissue only report the total loss of the compound over time. It is possible to determine k_m values by quantifying the changes in parent compound and metabolites (e.g., DBT and MBT) over time; however, this is rarely calculated.

7.5.3 Observations

Parental transfer of organotins is an important factor to consider, especially in light of the toxicity information for development. One study reported that maternal transfer of butyltins occurred for the Dall's porpoise; however, the concentrations in fetal tissue were relatively low compared to the adult (Yang and Miyazaki 2006). Concentrations of TBT in the fetus (1.0 kg at 6 months post fertilization) were about 10 times lower than maternal concentrations (15 ng/g vs. 1.4 ng/g, whole-body values determined by summation of organ burdens). Another study (Kajiwara et al. 2006) that examined organotins in stranded killer whales found similar differences (2–20 fold) for TBT concentrations in liver between mature adults and calves that were estimated to be a few months old. Of note were the concentrations of total phenyltins (tri- and di-), which were detected in all three calves and in only one of the five mature females, although the concentrations were low (≈1 ng/g).

The results for marine mammals are in stark contrast to those for fish as demonstrated for viviparous surfperch (*Ditrema temmincki*) (Ohji et al. 2006). The concentration of TBT in fry was 10–16 times higher than values reported for whole-body parental females. The percentage TBT in relation to total butyltins (TBT, DBT, and MBT) was 51% in the females and 81% in the fry indicating a reduced capacity for biotransformation. Due to early life-stage sensitivity of fish to butyltins in tissue, this is an important observation.

TBT bioaccumulation was also observed in algae. Maguire et al. (1984) reported a dry-weight TBT BCF for the green alga *Ankistrodesmus falcatus* of 30,000, which can lead to very high concentrations. The consequences of this high BCF value include the enhancement of dietary uptake by planktivores and direct toxicity to algal species because TBT is known to affect energy production in chloroplasts, heme metabolism, and disrupt ion pumps (Fent 1996).

The reason for the very high bioaccumulation factors for many species can be found in the rates of uptake and elimination. Many QSAR models have been developed for organic compounds

that relate K_{ow} with k_1 and k_2 (Connell 1990). These models are generally accurate predictors for passive organic-compound flux in species that exhibit low rates of metabolism. The toxicokinetic model for chlorobenzenes in molluscs was selected as an example (Connell 1990), because low metabolism was expected for this taxa. Based on the K_{ow} for TBT, the predicted values are 280/d for k_1 and 0.96/d for k_2. Using these toxicokinetic values, the predicted BCF is 292, which is very similar to the predicted BCF of 377 that was described earlier using the bioaccumulation QSAR equation for substituted chlorophenols. Measured values for k_1 and k_2 in species that are expected to exhibit low metabolic rates for TBT are generally substantially different than these predicted values. For example Gomez-Arizas et al. (1999) determined the TBT k_2 for clams (*Venerupis decussata*) to be approximately 0.02–0.03/d, which was similar to the values reported by Meador (1997) for an amphipod and Tessier et al. (2007) for a gastropod (*Lymnaea stagnalis*) (each k_2 = 0.04/d). These values are 25–50 fold lower than the expected elimination rates for passive diffusion. The QSAR predicted k_1 value is 280/d, which is approximately 3–27 times less than other reported values (Meador 1997, Gomez-Ariza et al. 1999, Tessier et al. 2007). Given these large differences in predicted and observed toxicokinetic rates, it is not surprising that BCFs are far higher than those predicted with QSARs.

The observed TBT BCFs can be two orders of magnitude or more above predicted levels, which is consistent with the large observed disparity in toxicokinetics. Based on an examination of the limited available data, it appears that the k_2 rate constant exhibits a greater influence than the k_1 value as determinants for the BCF. We can conclude from this that TBT (and likely other organotins) is very slowly eliminated from tissue. Of course, for those species that are able to metabolize TBT, the overall elimination rate will be higher; however, the passive rate of elimination is still an important factor for determining k_2 and bioaccumulation factors. Also noteworthy is that the k_2 values in this range (0.02–0.04/d) indicate that some species will take ≈75–150 days to reach steady-state tissue concentrations.

7.6 ORGANOTIN TOXICITY

In all cases an organotin compound is far more toxic than its individual components. For example, the toxicity of TBT is considerably more toxic than inorganic tin or the component butyl groups. For comparison, this is generally the same pattern for organomercurials, but not for arsenic because methylation reduces toxicity and the inorganic forms tend to be more toxic. Within a series of organotin compounds there are differences in toxicity. When expressed in terms of water exposure, the triorganotins (e.g., TBT, TPT, TMT, and TPrT) are considered more toxic than the mono-, di-, or tetraorganotins (Laughlin et al. 1985, Brüschweiler et al. 1995). These authors proposed that the increased water toxicity for the triorganotin compounds may be a result of several factors such as their higher K_{ow} values, a higher rate of uptake, differences in the MeOA, or the increased propensity for bioaccumulation and persistence.

Many of the factors that should be considered during toxicity assessment from ambient exposure are less important for tissue-residue toxicity. For most contaminants, pH, redox state, organic carbon, and salinity are controlling factors for ambient-toxicity metrics; however, once we consider tissue concentrations these factors are considerably less important. The factors that are important for both ambient-exposure and tissue-residue toxicity metrics include organism health, temperature, and lipid content (Meador et al. 2008). Lipid content is an important parameter for hydrophobic compounds because of internal toxicant partitioning and the relative amount of the active toxicant fraction (i.e., biologically effective dose) (Lassiter and Hallam 1990). There are few data on this subject for organotins; however, one study found that the LR_{50} (lethal tissue residue) for an amphipod (*Rhepoxynius abronius*) was approximately three times lower in individuals containing a reduced lipid content (Meador 1993). When this toxicity metric was normalized to lipid content and expressed on a lipid basis the values for the normal and reduced lipid groups became statistically indistinguishable.

7.6.1 Toxicity from Ambient Exposure

It is well known that the toxicity of organotins varies widely among compounds and species when external exposure (e.g., water concentrations) is considered. Water exposure to TBT produces LC_{50} values ranging over two orders of magnitude among aquatic species (~0.5–200 ng/mL) (Figure 7.1) and three orders for most sublethal responses such as growth and reproductive impairment (0.005–5 ng/mL) (Cardwell and Meador 1989, Meador 2000, U.S. EPA 2003).

One comprehensive study examined the aqueous toxicity of seven diorganotins (R_2SnX_2) and eight triorganotins (R_3SnX_2) to crab zoeae (*Rhithropanopeus harrisii*) (Laughlin et al. 1985). The R groups for the triorganotins were methyl, ethyl, propyl, butyl, phenyl, and cyclohexyl. The same list applies for the diorganotins with the addition of benzyl. All of the X groups were oxides, hydroxides, bromides, and chlorides. The X group is essentially unimportant because as soon as the compound is added to water it speciates according to the pH, redox state, and the ionic content of the receiving water. The variability among diorganotins for the day 14 LC_{50} was 250 fold and for the triorganotins was 28 fold. For all compounds the range was four orders of magnitude, which was very similar to that reported by Nagase et al. (1991) who determined the 48 h LC_{50} for killifish (*Oryzias latipes*) exposed to 29 different organotins (Figure 7.1). In the Laughlin et al. (1985) study, a strong linear relationship was found between LC_{50} and the Hansch lipophilicity parameter (π) for both the di- and triorganotins ($r^2 > 0.94$) indicating that the most important factor determining toxicity was the hydrophobic characteristics of each compound. We would expect differences in potency among these compounds when toxicity is based on tissue concentrations; however, when considering ambient-exposure toxicity metrics, the variability due to differences in toxicokinetics

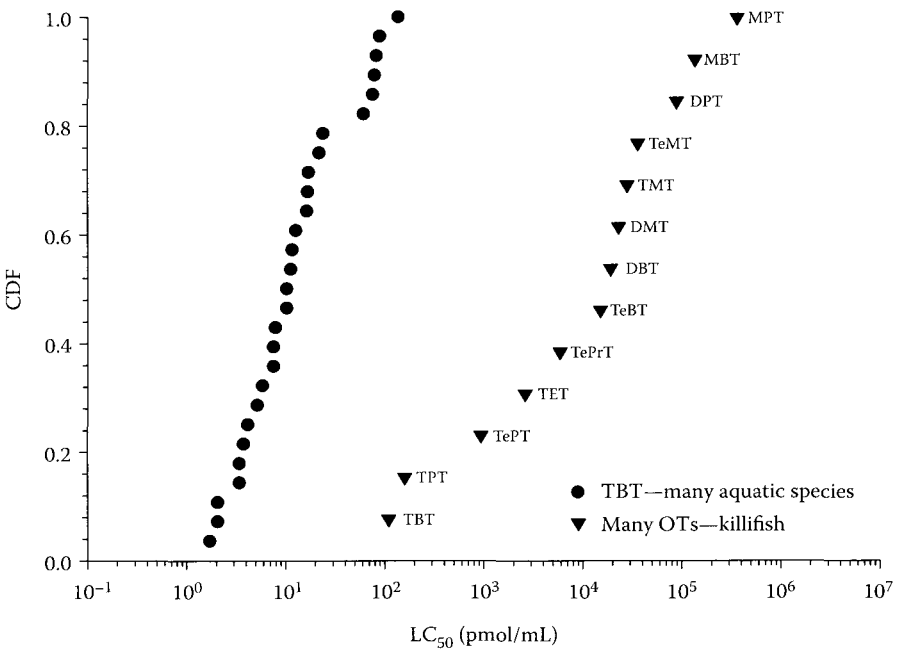

FIGURE 7.1 Circles show 96 h LC_{50} values in pmol/mL for 28 aquatic species (polychaetes, amphipods, copepods, shrimp, and fish) exposed to TBT in water. Data from Cardwell and Meador (1989). Triangles are the 48 h LC_{50} values for one species (killifish, *Oryzias latipes*) exposed to many different organotins (OTs) (Nagase et al. 1991). Of the 29 organotins tested on killifish, 16 were unique (many were salts of one compound). Only three were not shown (*n*-butyltrimethyltin, di-*n*-butyldimethyltin, and tri-*n*-butylmethyltin) all of which exhibited LC_{50} values that fell within the range shown. See Table 7.1 for organotin abbreviations. CDF is cumulative distribution function.

(bioaccumulation) versus that for toxicodynamics (potency) can not be distinguished. As shown later, the differences in potency are relatively minor among all these organotins for lethality.

This observation is supported by an example for DBT and TBT, both of which are known to inhibit adenosine triphosphate (ATP) synthesis. One study found that DBT was more than 50 times less toxic than TBT (Lytle et al. 2003) when based on exposure (water) concentrations; however, another study demonstrated that DBT was only three times less potent than TBT for this MeOA when based on tissue concentrations (Aldridge et al. 1977). These results highlight the differences between aqueous and tissue-residue toxicity metrics among compounds as a function of bioavailability and external toxicokinetics. Based on this information, the diorganotins will likely cause lethality in aquatic species at tissue concentrations that are relatively similar to those determined for TBT lethality.

Another study on organotin toxicity is useful for highlighting the role of bioaccumulation and QSARs. Vighi and Calamari (1985) reported the 24 h acute LC_{50} values for *Daphnia magna* exposed to TMT, TET, TPrT, TBT, and TPT. These values ranged from 13 to 470 ng/mL, which is a factor of 36. This range in toxicity values is relatively minor compared to the approximately six orders of magnitude range for compound K_{ow} values and the expected differences in bioaccumulation as predicted by K_{ow} QSARs. One conclusion from these data is that the predicted BCF values do not accurately reflect the observed values. Based on the highly similar tissue-based mortality data presented here (Tables 7.3 and 7.4) it is

TABLE 7.3
Critical Triorganotin Body Residues for Aquatic Species

Response	Organotin	Species	CBR (nmol/g)	Range (nmol/g)	SD	CV (%)	CBR (ng/g)	Range (ng/g)	*n*
Mortality	TBT, TPT	Fish and invertebrates	33.4	12–51	12.4	37	9600	3500–14,780	11
Growth impairment	TBT	Fish and invertebrates	2.1	0.56–4.3	1.2	57	640	162–1246	11
Growth stimulation/ obesogen	TBT	Fish	0.04	—	—	—	13	—	1
Behavior	TBT	Fish and invertebrates	0.70	0.35–1.0	—	—	200	100–300	2
Imposex—female sterilization	TBT, TPT	Gastropod snails	0.29	0.05–0.42	0.21	73	85	14–141	11
Imposex—threshold	TBT, TPT	Gastropod snails*	0.10	0.03–0.17	0.06	62	30	10–49	4
Reproductive impairment	TBT, TPT	Invertebrates	0.48	0.006–0.97	0.49	100	140	2–280	3
Reproductive impairment	TBT, TPT	Fish—adult/ juvenile	0.07	0.06–0.08	0.01	20	24	18–29	2
Reproductive impairment	TBT, TPT	Fish—egg	0.28	0.01–0.55	0.23	81	83	5–160	5

Mean, standard deviation (SD), and range in values for critical body residues (CBRs) in nmol/g wet weight and equivalent ng/g value. Mortality CBR is based on LR_{50}. The CBRs for reproductive impairment, growth impairment, and growth stimulation based on LOER and ERp values. The imposex CBR for female sterilization based on definition in Meador et al. (2002). Range shows minimum and maximum values for n studies, most of which are for different species. CV is the coefficient of variation in %. Values are for whole body, except (*), which are whole body or muscle tissue. Last row shows concentrations for eggs. Data from this chapter, Meador (2000), Meador et al. (2002), Meador (in press).

Source: From Meador, J. P., *Rev. Environ. Contam. Toxicol.*, 166, 1–48, 2000; Meador, J. P. et al., *Aquat. Conserv.: Mar. Freshwat. Ecosyst.*, 12, 539–551, 2002. With permission.

TABLE 7.4
Lethal Values for Several Organotins in Small Mammals

Triorganotins	Species	LR_{50} i.p. µg/g	SD	nmol/g
Trimethyltin	Rat	16	—	97.6
Triethyltin	Rabbit	10	—	48.8
Triethyltin	Rat	10	—	48.8
Tributyltin	Rat	10	—	34.6
Triphenyltin	Rat	11.4	(2.0)	32.3
Triphenyltin	Guinea pig	3.7	—	10.7
Triphenyltin	Mouse	7.9	—	37.1
Triphenyltin	Rabbit	16	—	97.6
Tricyclohexyltin	Rat	13	—	34.7
Trioctyltin	Rat	>48	—	>100
Diorganotins	**Est. metric**	**Est. LR_p µg/g**		**nmol/g**
Dimethyltin	LR_{50}	40	—	270.3
Diethyltin	LR_{100}	40	—	226.0
Dipropyltin	LR_{75}	10	—	48.8
Di-isopropyltin	LR_{100}	20	—	97.6
Dibutyltin	LR_{100}	10	—	42.9
Dipentyltin	LR_{100}	20	—	73.5
Diphenyltin	LR_{100}	15.3*	—	56.0
Dihexyltin	LR_{50}	10	—	33.6
Dioctyltin	LR_{100}	10	—	28.2

Calculated LR_{50} values for triorganotins based on intraperitoneal (i.p.) injection. Each diorganotin tested at four doses by intravenous injection (i.v.) to groups of four rats. For diorganotins, LR_p (p for percentage) was estimated by the reported number of mortalities per treatment. The TPT LR_{50} for rat based on four experiments. Mean and standard deviation (SD) for all triorganotin LR_{50} values (except trioctyltin) is 10.9 (3.9) µg/g. * Is i.p. injection. Data from Barnes and Stoner (1958), Stoner (1966), and Kimbrough (1976). See text for details.

likely that the mode or MeOA for mortality is the same for all these organotins, which would reduce the importance of potency as a factor in the observed disparities in LC_{50} values and expected BCFs.

7.6.2 Responses and Modes and Mechanisms of Toxic Action

Toxic response can be considered at three levels. The first level is the organismal response (e.g., mortality, growth, and reproduction), the second level is mode of action (MoOA), and the third is the mechanism of action (MeOA). Organotins are known to cause several adverse effects including growth impairment, growth enhancement, abnormal development, altered behavior, and reproductive effects. The terms "mode and mechanism of toxic action" have distinct meaning (Meador et al. 2008). In general, the mode of action is the higher level of toxicological disturbance to biological function consisting of physico-chemical, physiological, or biochemical pathway alterations resulting from one or more MeOAs. Triorganotins, and some of the disubstituted organotins, are known to act by several MoOAs including inhibition of cellular energy metabolism (Aldridge et al. 1977, Hunziker et al. 2002), endocrine disruption (Matthiessen and Gibbs 1998, Grün et al. 2006), neurotoxicity (Walsh and DeHaven 1988), inhibition of ion pumps (Fent 1996), inhibition of cytochrome

P450 (Fent 1996), inhibition of intracellular enzymes (Walsh and DeHaven 1988), and immune system impairment (Bouchard et al. 1999, De Santiago and Aguilar-Santelises 1999). There is very little toxicity information for the monosubstituted organotins.

Most, if not all, of these MoOAs likely result from multiple MeOAs (the crucial and specific biochemical alteration) or inhibition of specific pathways. Several definitions for MeOA have been proposed; however, in many applications it refers to the biochemical target or specific biochemical pathway affected. More precise definitions have recently been proposed for both mode and MeOA (Borgert et al. 2004, Meador et al. 2008). For example, there are many compounds that are considered uncouplers (a mode of action), but do so by different biochemical mechanisms. This distinction is important, especially when considering the nature of toxicant interactions (e.g., additivity [dose or response] and those that are less or more than additive).

We have some information regarding toxic action for the commonly encountered organotins and limited information for most of the other compounds. There are some similarities among the organotins in the MeOA, especially for the acute mortality response for triorganotins. For the sublethal response, there are a variety of MoOAs and MeOAs for organotins, which are likely a function of dose and the compound's unique stereochemistry and resultant association with biomolecules. Detailed work by a few authors discovered that several of the triorganotins (TMT, TET, TBT, TPT, and Tri-c-hexyltin [TcHT]) and many of the diorganotins (DMT, DET, dipropyltin [DPrT], DBT, DPT, and dihexyltin [DHT]) inhibit respiration (Aldridge 1958, 1976, Aldridge et al. 1977, Connerton and Griffiths 1989). In addition, these diorganotins block the pathway from glutamate to oxoglutarate as part of the Krebs cycle in mitochondria (Aldridge 1976). Tetraorganotins generally do not inhibit respiration (Aldridge 1976); however, once bioaccumulated they are metabolized to triorganotins by many species.

Several recent studies have explored the role of organotins as agonists of nuclear hormone receptors and their role as endocrine disruptors and obesogens (Grün et al. 2006, Grün and Blumberg 2007). One study demonstrated that TBT and TPT were potent activators of the retinoid X receptor (RXR) and peroxisome proliferator-activated receptor (PPARγ) (Grün et al. 2006) at low concentrations (3–20 pmol/g). They also showed that several other butyltins were activators of these receptors but at higher levels; tetrabutyltin (TeBT) (150 pmol/g), DBT (3000 pmol/g), TET (2800 pmol/g), and TMT (>10,000 pmol/g). MBT was not active. These very low potency factors for RXR were confirmed by Hu et al. (2009) for TBT and TPT, which were essentially identical (9.6 and 20 pmol/g, respectively). According to Grün et al. (2006) some organotins (especially TBT and TPT) are potent endocrine disruptors that target adipogenesis by modulating key regulatory transcription factors via RXR and PPARγ.

Specific organotins can act by multiple MoOAs and MeOAs that are likely dose and time dependent. For example, short-term exposure to high doses of TBT leads to mortality and growth inhibition; however, under chronic low-dose exposures endocrine and immunotoxic responses can be observed in a variety of species. For these, and many compounds, it is important to consider critical toxic concentrations among species for a given response, not all responses combined.

7.6.3 Tissue-Residue Toxicity

The following is a brief survey of the various biological responses reported for organotins. For aquatic species, most of the focus has been on TBT and its effects on survival, growth, and reproductive impairment and there are limited data for TPT. In addition, there are toxicity data for several of the tri- and diorganotins for small mammals that are informative. Unfortunately, there are no tissue-residue mortality data for aquatic species exposed to di- or monoorganotins.

7.6.3.1 Mortality

In terms of the tissue-based toxic response, the TBT concentration causing lethality is approximately 100 times less than that for baseline (narcosis) toxicants that has been characterized for a large number of organic compounds (Di Toro et al. 2000). As shown in Table 7.3, sublethal toxic responses can

occur at tissue residues that are 700 times lower than those for lethal levels. The lethal concentration for some organotins has been determined in several species and the values are remarkably consistent. One comparative study with guppies (*Poecillia reticulata*) found very similar lethal tissue concentrations for TBT, TPT, and TcHT, although tri-*n*-hexyltin (TnHT) was about 10 times more toxic ($LR_{100} \approx 1$ nmol/g ww) on a tissue-residue basis (Tas 1993). It is important to note here that most of the mortality data are based on short-term exposures (acute). It is possible to observe mortality during chronic exposure, which may be associated with far lower tissue residues. These chronic mortalities are likely a secondary response to the primary effect, such as mortality from a pathogen due to a weakened immune system or starvation from the inhibition of energy producing pathways.

The mortality values in Table 7.4 were determined by intraperitoneal (i.p.) or intravenous (i.v.) injection (as μg organotin injected per gram organism), which were used to estimate the LR_p values. Dosing by injection produces far less variable toxicity metrics than what is generally obtained when these compounds were administered orally (administered dose) to produce an LD_p or ED_p value. The variability in metabolism among species can have a large impact on the LD_{50} when toxicants are introduced orally, which is likely mitigated when using the injection route of exposure. With injection, the toxicant is quickly distributed to the tissues and results in a response when the critical concentration is achieved. Because tissue-residue toxicity metrics are often time independent for many toxicants (Meador 2006), this route of exposure would lead to a reasonable estimate of the tissue (e.g., whole-body) concentration (acquired dose) associated with the biological response (i.e., the LR_p or ER_p). The type of response is also important when considering injection as the route of exposure. These results may be reliable for the acute (short term) lethality response, but less so for sublethal responses that require extended periods of time at relatively constant concentrations to develop and manifest.

The literature on small mammals indicates a remarkable similarity in the LR_{50} values for several triorganotins. Data were found for nine toxicity values from tests with five organotins (TMT, TET, TBT, TPT, and TcHT) and four common laboratory bioassay species. The mean (SD) LR_{50} for all species and triorganotins was 11.0 (3.6) μg/g whole-body ww (Table 7.4). Interestingly, this value is almost identical to the TBT LR_{50} in Table 7.3 for 10 species of fish and invertebrates. Based on the data in Table 7.4 it is likely that these triorganotins act by the same MoOA (and possibly mechanism), which is assumed to be uncoupling of oxidative phosphorylation. Interestingly, the LR_{50} for trioctyltin was far above 48 μg/g via i.p. injection (no response at this concentration) (Table 7.4) (Barnes and Stoner 1958), and this is the one triorganotin that is considered not to be an uncoupler. The low variability for these data also imply that the whole-body tissue distribution for the various routes of exposure (injection, ingestion, and ventilation) result in similar internal tissue partitioning, which may not be the case for other toxicants.

Barnes and Stoner (1958) report lethal toxicity data for rats exposed to several diorganotins (methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl, and octyl) that were administered via i.v. injection. Although sample sizes were small (four animals per dose, four doses for each diorganotin), all these diorganotins caused 50–100% mortality within 2–72 h at doses between 10 and 20 μg/g, except for DMT (50% mortality at 40 μg/g) and DET (100% mortality at 40 μg/g). Interestingly, dioctyltin (DOT) was relatively toxic, especially compared to trioctyltin. Because trioctyltin (TOT) is a large and presumably very hydrophobic compound (log K_{ow} for DOT is 5.8 and likely much higher for TOT) it may exhibit steric hindrance for membrane permeability. As mentioned previously, several of the diorganotin compounds are known inhibitors of energy production and because these compounds resulted in mortality at similar concentrations as the triorganotins, it is likely that they also act by the same MoOA or MeOA.

In general, we have very little tissue-residue-based lethality data for organotins other than triorganotins in aquatic species; however, we can estimate the acute toxicity for a methyltin. As described in Meador (2006), multiplying the BCF by the LC_{50} for time-matched values will result in the LR_{50}. A study by Hadjispyrou et al. (2001) provided the 24 h BCF and LC_{50} for the brine shrimp *Artemia franciscana* exposed to DMT. Using this equation, the resulting LR_{50} value is 78 μg/g ww, which

is only 1.95 times higher than the value in Table 7.4 for rats. Hadjispyrou et al. (2001) also provide data for TMT; however, the BCF was determined at a concentration far higher than the LC50 and could not be used for this estimation.

Surprisingly, the lethal toxicity for TBT and TPT are very similar among invertebrates, fish, and mammals (Figure 7.2, Table 7.4). As seen in this figure, the LR_{50} for small mammals exposed to TBT via intravenous or intraperitoneal injection is almost identical to the observed LR_{50} for fish and invertebrates exposed via water or diet with most values between 10 and 50 nmol/g. Considering the 28-fold variability in aqueous LC_{50} values reported by Laughlin et al. (1985) and the 1800-fold range by Nagase et al. (1991) for one species exposed to triorganotins, this range of fivefold for tissue-residue-based mortality for five triorganotins in a very wide diversity of taxa is very low. Based on the similarities in the tissue-based mortality metrics for mammals, fish, and invertebrates exposed to triorganotins and the fact that most tri- and diorganotins are considered uncouplers, a reasonable assumption would be that the diorganotins would also result in similar acute lethality toxicity metrics for aquatic species. An important conclusion from these data is that all these compounds (di- and triorganotins) are likely dose additive for this response and all species considered.

7.6.3.2 Growth

Several studies have demonstrated reduced growth in a variety of aquatic species exposed to TBT (Table 7.3). Most of those studies show that growth inhibition occurs at a relatively consistent concentration of approximately 2 nmol/g (0.6 μg/g ww) for whole body (Meador 2000, 2006). A reasonable hypothesis for this response is that inhibition of oxidative phosphorylation causes a reduction in the available energy needed for growth; however, we do not know the actual MoOA

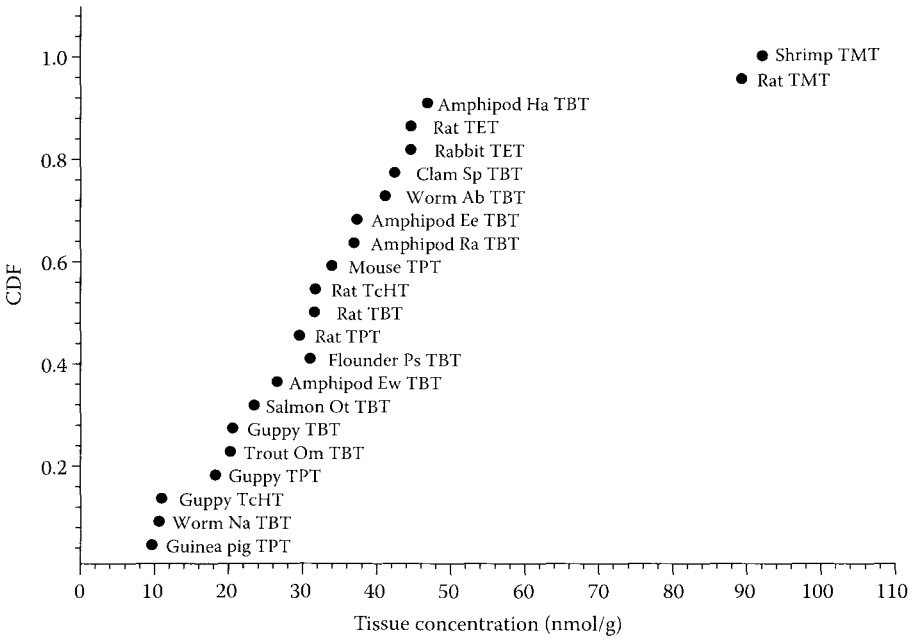

FIGURE 7.2 Values are whole-body lethal concentrations (LR_{50}) for 15 species and five organotins. The small mammal values are equivalent to the intraperitoneal injection concentration. Values range from 10.6 to 100 nmol/g wet weight and species names are centered on the value (e.g., guinea pig TPT is 10.6 nmol/g). Species are shrimp (*Artemia franciscana*), amphipods (*Hyalella azteca*; *Eohaustorius estuarius*; *E. washingtonianus*; *Rhepoxynius abronius*), clam (*Scrobicularia plana*), polychaete worms (*Armandia brevis*; *Neanthes arenaceodentata*), flounder (*Platichthys stellatus*), salmon (*Oncorhynchus tshawytscha*), and trout (*O. mykiss*). See Table 7.1 for organotin abbreviations. CDF is cumulative distribution function.

or MeOA for this response. As discussed later, organotins are known to affect metabolism and adipogenesis and low concentrations are known to affect growth, therefore this response may be due to long-term disruption in the pathway for steroid metabolism or the result of low-level effects on energy production in mitochondria. Widdows and Page (1993) found an impact to the Scope for Growth metric (joules/hour) at a TBT tissue concentration of ≈1 μg/g in mussel (*Mytilus edulis*). This value represents the difference between the energy absorbed from food and the energy expended with respiration.

There are very few studies that demonstrate concordance between lab and field-toxicity data, which has been confirmed for TBT. The bioaccumulation and toxicity data from field studies for TBT are remarkably similar to the values determined in the lab (Salazar and Salazar 1995, 1998). The tissue concentration shown to be associated with impaired growth in mussels from caging studies in contaminated harbors is approximately 0.8 μg/g (wet wt.), which is essentially identical to the value in Table 7.3 that is derived from lab studies.

TPT is also known to affect growth; however, none of the studies examined reported tissue concentrations for this response. Rehage et al. (2002) described reduced growth in larval salamanders exposed to 1 ppb of aqueous TPT, which may have resulted in a relatively low tissue concentration depending on the toxicokinetics for this species. In addition, Stoner (1966) reported a reduction in body mass for guinea pigs fed a relatively low dose of TPT (1 μg/g in diet ≈ 0.1 μg/g bw/day).

Another well-known effect due to TBT exposure is the chambering response in bivalves. Many laboratory (Chagot et al. 1990, Coelho et al. 2006) and field studies (King et al. 1989, Alzieu 2000) have documented that low exposure concentrations of TBT (5–100 ng/L; approximately 10–100 ng/g in tissue) resulted in excessive shell growth (chambering) in oysters and clams. King et al. (1989) found a strong correlation between the number of shell chambers for *Crassostrea gigas* and TBT tissue concentrations with an apparent threshold of ≈100 ng/g ww. This abnormal growth severely impairs the marketability for oysters and may result in reproductive impairment because a large percentage of the available energy is utilized for shell growth leaving less for somatic and reproductive biomass. This response may be considered a growth effect; however, in some cases the shell malformation is observed at concentrations lower than those causing reductions in soft-tissue biomass.

7.6.3.3 Immunotoxicity

This mode of action for organotins may result in several biological changes including atrophy of the thymus, reduction of kidney macrophages, and changes to the spleen (Fent 1996). One of the biochemical mechanisms promoting immunotoxicity is likely related to the disruption of calcium homeostasis (Chow et al. 1992). The human health reference dose for TBT (0.3 ng/g bw/d) is based on immunotoxicity (U.S. EPA 1997), and there is evidence that DBT is also immunotoxic (Fent 1996, O'Halloran et al. 1998). Bouchard et al. (1999) and O'Halloran et al. (1998) concluded that DBT was a more potent immunotoxicant than TBT (based on concentrations in hemolymph and cell cultures), which had important implications for assessing the toxicity of DBT tissue concentrations. When considering organotin tissue residues, DBT is particularly important because of its immunotoxic potency and because high concentrations of DBT are often found in tissue as a result of TBT metabolism. The metabolic conversion of organometallics to other compounds and their often toxic nature provides a strong argument for considering both the parent compound and any metabolites when conducting a toxicity assessment based on tissue residues.

7.6.3.4 Reproductive

7.6.3.4.1 Imposex in Molluscs

Some organotins are also potent endocrine disruptors causing imposex in meso- and neogastropods (Matthiessen and Gibbs 1998), which is the manifestation of secondary male sexual characteristics in female gastropods. The imposex abnormality was the primary driver for the TBT water quality

criteria promulgated by the U.S. EPA (2003) because this response occurred at the lowest effect concentrations. For aquatic organisms, this reproductive impairment is one of the most sensitive responses and it also occurs at the lowest tissue concentrations (Meador 2006). The observation of imposex in molluscs at relatively low tissue concentrations is supported by laboratory (Bryan et al. 1988, Horiguchi et al. 1997a, 1997b) and field studies (Horiguchi et al. 1994, Morcillo and Porte 1999, Barreiro et al. 2001, Bech et al. 2002). The threshold for imposex in snails has been reported to occur in the 10–50 ng/g ww range (Table 7.5).

Several theories have been offered over the years on the mechanism for imposex in stenoglossan snails; however, the actual mechanistic response was described recently by Nishikawa et al. (2004). As described in this study, TBT and TPT are potent agonists for the RXRs. In addition to demonstrating that TBT strongly binds RXRs, the authors of this elegant study were able to induce imposex in the rock shell (*Thais clavigera*) within 4 weeks by injecting individuals with 9-cis retinoic acid, the natural ligand for RXR.

TBT is the main focus for imposex studies; however, TPT also causes imposex within the same range of tissue concentrations (Table 7.5; Horiguchi et al. 1997a). Given the similarity in the dose–response relationship for imposex and these two compounds they should be considered additive when assessing imposex. The concentration of TBT and TPT in tissue associated with sterilization in these molluscs occurs at approximately 85 ng/g ww (Meador et al. 2002; Table 7.3). Sterilization for these molluscs is a severe effect and has been linked with adverse population level attributes (Oehlmann et al. 1996, Horiguchi et al. 1997b).

One paper reported the results for six organotins (TBT, DBT, MBT, TPT, DPT, and MPT) and their potential to cause imposex in *Thais clavigera*, a commonly studied snail for this response (Horiguchi et al. 1997a). A sequential series of experiments using high tissue doses (via injection) to screen organotins and lower doses to characterize the degree of increased penis length in female snails found that the triorganotins TBT, TPT, and TPrT were the strongest inducers of imposex. This study also concluded that the di- and monoorganotins (DBT, MBT, DPT, and MPT) did not cause the response. Bryan et al. (1988) also concluded that DBT did not lead to imposex, and they observed mixed results for TPrT (positive results only at high concentrations, i.e., >500 ng/g ww) in the snail *Nucella lapillus*. They also reported no response to TPT when animals were exposed to 590 ng/L in water or injected with 4.4 µg/g bw. TeBT was also tested by Bryan et al. (1988); however, they concluded that the observed positive response may have been caused by TBT contamination, which is commonly found in stock solutions.

A recent study reported a high correlation ($r^2 = 0.94$) between the degree of imposex in snails and the extent of DNA damage as assessed with micronucleus formation in hemocytes (Hagger et al. 2006). This study also found a high correlation between whole-body TBT concentrations and neoplastic proliferations on genital organs of male and female snails.

7.6.3.4.2 Fish Reproduction and Development

At least five studies examined early-life stage effects in fish due to the exposure of eggs to TBT. Three of the studies assessed the effects of maternally transferred TBT using different routes of exposure including dietary (Nakayama et al. 2005, Shimasaki et al. 2006) and aqueous (Zhang et al. 2008). Two other studies exposed the eggs via nanoinjection (Hano et al. 2007, Hu et al. 2009). A number of adverse effects were reported in these studies, and many of those responses occurred when TBT was approximately 5–160 ng/g ww egg (Table 7.5). Based on these five studies, we can conclude that fish embryos are very sensitive to TBT at these low concentrations and that maternal transfer is an important route of exposure.

Reproductive effects were also observed in juvenile and adult fish at very low tissue concentrations. A high rate of sex reversal was observed among genetic female flounder (*Paralichthys olivaceus*) at a whole-body TBT concentration of 18 and 160 ng/g ww (Shimasaki et al. 2003). These authors also reported a statistically significant decrease in growth (body weight and length) at 18 ng/g ww. Other studies on reproductive effects in adult fish include Zhang et al. (2008) who

TABLE 7.5
Tissue Concentrations Associated with Reproductive or Early-Life Stage Responses by Aquatic Species to Tributyltin (TBT) and Triphenyltin (TPT)

	Responses	Species	Type	ng/g	Tissue	nmol/g	Exposure	Reference
			Fish					
TBT	↓Viable hatch, ↓viable larvae, ↓floating egg rate	*Sillago japonica*	Whiting	85–160	Egg	0.30	Maternal lab	Shimasaki et al. (2006)
TBT	↓Fertility, ↓hatchability, ↓swim up rate	*Oryzias latipes*	Medaka	<20–265	Egg	0.07–0.9	Maternal lab	Nakayama et al. (2005)
TBT	↓Swim up rate, ↑mort	*O. latipes*	Medaka	160	Egg	0.55	Nano injection	Hano et al. (2007)
TBT	↓Growth, ↑%male	*Paralichthys olivaceus*	Japanese flounder	18	Whole body	0.06	Dietary lab	Shimasaki et al. (2003)
TPT	↓Protein/egg, ↓hatching success, ↓swim up success, ↓surviving larvae/female/d, ↑hemorrhaging, ↑abnormal ocular development	*O. latipes*	Medaka	4.6	Egg	0.013	Maternal lab	Zhang et al. (2008)
TPT	↑Abnormal ocular development	*Acipenser baerii*	Sturgeon	27	Egg	0.08	Nanoinjection	Hu et al. (2009)
TPT	↓Vitellogenin, ↓spawning frequency, ↓eggs/female/d	*O. latipes*	Medaka	29	Whole body	0.08	Lab	Zhang et al. (2008)
			Invertebrates					
TBT	↑Male repro cells in ovary	*Haliotis gigantea*	Abalone	2.4	Muscle	0.008	Lab	Horiguchi et al. (2002)
TBT	Imposex—100% females	*Bolinus brandaris*	Snail	141	Whole body	0.49	Field	Morcillo and Porte (1999)
TBT	↑Imposex	*Thais distinguenda*	Snail	10	Whole body	0.03	Field	Bech et al. (2002)
TBT	↑Imposex	*Nassarius reticulatus*	Snail	35	Whole body	0.12	Field	Barreiro et al. (2001)
TBT	↑Imposex	*Hydrobia ulvae*	Snail	49	Whole body	0.17	Field	Schulte-Oehlmann et al. (1998)
TBT	↑Imposex	*Thais clavigera*	Snail	20	Whole body	0.07	Lab	Horiguchi et al. (1997b)*
TBT	↑Imposex	*Nucella lapillus*	Snail	330	Whole body	1.1	Lab	Bryan et al. (1988)
TBT	↑Imposex	*T. clavigera*	Snail	221	Whole body	0.76	Lab	Horiguchi et al. (1997a)
TBT	↓Number of young (50% decline; EC_{50})	*Hyalella azteca*	Amphipod	284	Whole body	0.98	Lab	Bartlett et al. (2004)
TPT	↑Imposex	*T. clavigera*	Snail	44	Whole body	0.13	Lab	Horiguchi et al. (1997a)
TPT	↑Male repro cells in ovary	*H. gigantea*	Abalone	126	Muscle	0.44	Lab	Horiguchi et al. (2002)

Values (as wet weights) are from recent laboratory studies or field assessments. * Details for this study in Horiguchi (1993). All responses exhibited statistical p-values ≤ .05 at the stated tissue concentration, which is usually the lowest observed effect residue (LOER).

reported decreased vitellogenin, spawning frequency, and eggs/female/day at very low whole-body concentrations (29 ng/g ww).

Another study on fish reproductive effects due to TBT exposure found significant increases in the sex ratio (males:females) and percentage abnormal sperm in zebrafish (*Danio rerio*) exposed from hatch to day 70 at an aqueous concentration of 0.1 ng/L (McAllister and Kime 2003). They also reported a large and significant decrease in sperm motility at 1 ng/L and the complete absence of flagella in sperm at 10 ng/L. The typical long-term BCF for small fish range from 300 to 5000 (Fent 1996, Zhang et al. 2008). If we use the high BCF estimate of 5000 that was determined for medaka (Zhang et al. 2008), the whole-body tissue concentration in the zebrafish from the McAllister and Kime (2003) study is predicted to be 0.5 ng/g for the 0.1 ng/L exposure concentration and 5 ng/g for the 1 ng/L treatment. This conservative estimate for the BCF results in very low tissue concentrations for these adverse effects that are comparable with measured concentrations and adverse effects for fish reported in Table 7.5.

7.6.3.4.3 Reproductive Effects in Other Species

One study with the starfish (*Leptasteria polaris*) reported large and statistically significant reductions in the diameter of previtellogenic and mature (final-stage) oocytes in females and the thickness of gonadal epithelium for both sexes (Mercier et al. 1994). These responses occurred at TBT concentrations of ≈300 ng/g as measured in the pyloric caeca. Concentrations of TBT were below the detection limit (2.5 ng/g) in the gonads. Throughout this 53-day dietary exposure study the metabolites DBT and MBT continued to increase indicating metabolism of the accumulated TBT. The authors concluded that oocyte development could not be maintained at this tissue concentration due to the thinning gonadal epithelia and consequent lack of available nutrients for the oocytes to develop.

Another study with TBT and copepods found reproductive impairment at exposure concentrations of 10, 50, and 100 ng/L (Johansen and Mohlenberg 1987), which covers that range of aqueous concentrations usually reported for imposex in snails. These results indicate that reproductive effects at these low concentrations are not limited to gastropod snails. Because stenoglossan snails and oysters exhibit the highest BCF values, these copepods are likely exhibiting reproductive effects at similar tissue concentrations or lower to those observed for molluscs.

7.6.3.5 Neurological

Many of the butyltins are neurotoxic, an effect that has been described for small mammals. TET binds myelin with high affinity, and TMT causes cell death in the limbic system, neocortex, and sensory neurons, which is considered a unique pathology (Walsh and DeHaven 1988). Because TMT and TET are potent neurotoxicants, behavioral effects are also common for mammals and likely other vertebrate classes. Unfortunately, very few data exists for aquatic organisms; however, invertebrate neurons do not contain myelin, therefore, this mode of action may be less important for these taxa. TBT and TPT are not generally considered neurotoxic (Fent 1996); however, they do cause behavioral changes. One study examined brain transmitters in rockfish (*Sebastiscus marmoratus*) that were injected intraperitoneally with TBT and TMT. TMT stimulated dose-response increases in the neurotransmitters aspartate and γ-aminobutyric acid (GABA) at all doses injected (10, 100, and 1000 ng/g bw). TBT caused an increase only in GABA at the highest dose. Both organotins affected the N-methyl-D-aspartate receptor (NMDAR) signaling pathway and its components such as calmodulin and calmodulin-dependent kinase II at most doses but in different directions (TBT downregulated and TMT upregulated NMDAR and other genes in this pathway) (Zuo et al. 2009). Changes to the levels of these neurotransmitters can alter neurotransmission, and by extension, cause abnormal neuronal function.

7.6.3.6 Behavioral

Triebskorn et al. (1994) demonstrated alterations in behavior for rainbow trout at whole-body TBT concentrations of approximately 300 ng/g ww. At this concentration fish exhibited hyperactivity by

swimming farther and faster for extended periods than control fish and also exhibited more random orientation in tanks. For some organotins, behavioral changes may be due to neurotoxicity; however, for TBT and TPT these responses could also be a result of an energy imbalance and higher than normal metabolism, which may result in lethargy or hyperactivity. Another study reported behavioral effects related to reproduction for medaka at a dietary dose of 1 μg/g bw/d (Nakayama et al. 2004) and concluded that the observed reduction in fertilization success was a result of the behavioral alteration.

Another study on behavioral alterations reported hyperactivity and a complete reversal in phototaxis for *Daphnia magna* exposed to TBT at concentrations between 0.5 and 1.0 ng/mL (Meador 1986). The percentage of individuals that exhibited positive phototaxis increased gradually from 0% to 100% over 6 days at 0.5 ng/mL and at a faster rate for the 0.75 and 1.0 ng/mL treatments. The photopositive animals increased their antennular strokes (an indication of activity and swimming speed) to 5 strokes/sec compared to 2 strokes/sec for the controls. Based on the BCF reported for this species by Fent (1996), the predicted tissue concentration for these responses at the lowest dose (0.5 ng/mL) is approximately 100 ng/g ww. Alteration in behavior is usually important for organism survival in the wild, and these results are especially noteworthy because *Daphnia* spp. and other zooplankton rely on light gradients and contrasts for vertical migration and antipredator behavior.

7.6.3.7 Obesogen/Somatogen

Obesogens are compounds that affect metabolic physiology and can lead to increased body fat, but not necessarily total body mass. Somatogens are compounds that promote increases in body mass (usually muscle) and possibly fat content. These compounds are often endocrine disruptors, and they may act through the same mechanism. Organotins have been implicated as obesogens, via activation of RXR and PPARγ(Grün and Blumberg 2007). These are the same receptors that have been implicated in the imposex response in snails. Because compounds that act as obesogens affect adipogenesis, it appears that they not only affect endocrine systems that control sexual differentiation but also the biochemical pathways that regulate lipid metabolism and growth. This is a new area of research and most of the limited number of studies have been conducted with small mammals. One recent study (Meador et al. in press) with juvenile chinook salmon (10–20 g) found that whole-body TBT concentrations as low as 13 ng/g ww caused significant increases in fish weight, whole-body lipid content, and several physiological parameters measured in plasma (glucose, alkaline phosphatase, lipase, triacylglycerols, and cholesterol). These results were more consistent with a somatogen response rather than the metabolic syndrome associated with the obesogen response. These whole-body tissue concentrations are in the same range as those that are considered threshold values for the imposex response in snails and reproductive effects in fish. Considering the role of RXR for imposex for both TBT and TPT, there is a high probability that TPT will also elicit these metabolic abnormalities at similar tissue concentrations reported for TBT.

The growth response for TBT is a good example of "hormesis," which is characterized by low-dose stimulation and high-dose inhibition (Calabrese and Baldwin 2003). As seen in Table 7.3, there are several studies demonstrating reduced growth in various species at whole-body concentrations of 600 ng/g. In this case we do not know if two separate dose-dependent MeOAs are involved or if these responses are just a continuum for one MeOA that produces different results depending of the degree of receptor interaction and length of time for exposure.

Summary

As shown for many organotins, bioaccumulation values are not predictable using the standard QSAR equations. Because there is no reliable way to predict bioaccumulation, direct observation is the best method for determining tissue concentrations for toxicity assessment. Bioaccumulation can be predicted with toxicokinetic rates; however, chemical concentration

data from water, sediment, or diet would be required to estimate tissue concentrations and these are highly variable. Given the highly elevated BCF values observed for TBT and TPT, very high tissue concentrations are expected for low exposure concentrations. Due to the highly toxic nature of these compounds at relatively low tissue concentrations, these organotins warrant the high level of environmental concern they have been given. Many of the other organotins are also very toxic at relatively low tissue concentrations; however, data on their occurrence in a variety of species are lacking therefore precluding a complete assessment of their potential toxicity. Even though BCF values are relatively low for some of these organotins (e.g., TMT and DMT), dietary uptake may be important for some. Many of the organotin compounds have not been detected in field-collected water or sediment and they are rarely analyzed in tissue. Some of the issues involve detection limits and a lack of studies to determine if these compounds occur. The butyltins and phenyltins are extensively studied and several tissue values have been reported for field-collected organisms. Except for the one study on seafood species (Forsyth and Clerous 1991), there are no field data for organotins, other than those mentioned earlier.

Although most of the research has been on TBT and the imposex response, a number of other organotins appear to be very toxic. Most notable is TPT, which exhibits a similar BCF and has been demonstrated to cause imposex at comparable concentrations as TBT. On the basis of the data presented in Table 7.2, it is evident that butyltins and phenyltins can occur in high concentrations in aquatic biota and should therefore always be considered together when assessing this response. When examined in light of the available toxicity information presented in this review, we can conclude that many species of fish and invertebrates exhibit concentrations high enough to result in adverse biological effects when exposed to these two triorganotins.

Surprisingly, the acute lethality values for many of the triorganotins, TMT, TET, TBT, TPT, and TcHT, are very similar among a variety of species from polychaetes to small mammals (Tables 7.3 and 7.4, Figure 7.2). Lethal tissue concentrations for these organotins and species exhibit a relatively tight range of concentrations (10–100 nmol/g; 4–16 ppm) with a low variance. Based on the work of Aldridge (1958), Connerton and Griffiths (1989), and others, most triorganotins (with the exception of trioctyltin) are considered uncouplers of oxidative phosphorylation, which is the likely mode of action responsible for lethality.

Also noteworthy is that many of the diorganotins (DPrT, DBT, DPT, DHT, and DOT) produce lethal responses in rats at very similar concentrations to those observed for triorganotins (Table 7.4). Mortality likely results from respiratory uncoupling and it appears to occur at very similar whole-body concentrations as that reported for triorganotins. As a result of this similarity, these compounds may be dose additive for the mortality response. This information suggests that a lethality-based toxicity assessment for organotins in field-collected species consider the summed concentration of all these di- and triorganotins.

Although not stated precisely as such, the concept put forth by Paracelsus (1493–1541) that the dose makes the poison, is germane here. For TMT we know that the LR_{50} for the brine shrimp is approximately 16.5 μg/g, which is very similar to values for other triorganotins. Due the low K_{ow} (low bioavailability) and high LC_{50} for this compound (220 ng/mL), it may not be an important environmental contaminant unless elevated tissue concentrations occur via dietary uptake. Even though most of the triorganotins lead to mortality at a similar whole-body tissue concentration, it is important to assess those tissue concentrations and their potential to reach adverse levels in feral organisms when concentrations in the environment (water, sediment, and prey) are relatively low.

Because comparable TBT and TPT tissue concentrations lead to similar responses it appears that these compounds may act similarly at the molecular level and bind the same receptors. This hypothesis is supported by the almost identical potency observed for interaction with the RXR, which is linked to the MeOA for endocrine- and metabolic-related responses (reproductive and growth disorders). On the basis of the research presented, comparable tissue concentrations for TBT and TPT will likely result in similar response levels for mortality, growth stimulation and

inhibition, and imposex. As a result of these observations, dose additivity would be a reasonable hypothesis for these two compounds, which means their concentrations should be added together when assessing these toxicological impacts (Meador 2006).

Even though imposex in molluscs is considered the most sensitive response, several recent studies have shown that fish respond at similar concentrations of triorganotins as those causing imposex. As more research is conducted, it will likely become evident that these very low tissue concentrations are able to cause adverse effects in a variety of taxa. It is clear from the data that TBT, and likely TPT, are very potent endocrine disruptors and reproductive toxicants for snails as well as other species. Based on these data there is no reason to limit the analysis of reproductive effects only to stenoglossan snails, which has been the intense focus for several years. All species should be considered at risk for reproductive impairment at these low tissue concentrations (10–50 ng/g ww).

The critical body residue (CBR) data for mortality, growth impairment, and population sterility due to imposex (Table 7.3) were used recently to develop tissue and sediment quality guidelines (Meador et al. 2002, Meador 2006). The observed variance for each endpoint-specific mean was relatively low allowing the selection of a mean value that could be used to assess toxic impact in field-collected organisms. Based on the available data presented here, it appears that whole-body tissue concentrations in the low ppb range (10–50 ng/g ww) represent threshold levels for a variety of effects in all aquatic species and in many cases result in serious impairment. Higher concentrations (100–500 ng/g ww) should be considered toxic to all species and likely to cause adverse effects in individuals, and potentially populations, if the exposure is long term. Any tissue concentrations in the low μg/g range (1–10 ppm) should be considered lethal for all species.

For the well-studied organotins (TBT and TPT), the combination of high uptake kinetics and slow rates of elimination coupled with relatively high-potency results in very toxic environmental contaminants. This combination of high bioaccumulation and potency is why organotins are considered one of the most toxic anthropogenic compounds ever released into the environment. Given some of the similarities in bioaccumulation and potency for the other organotins discussed in this review, we can conclude that this class of compounds warrants caution, assessment, and action when found in feral organisms.

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REFERENCES

Aldridge, W. N. 1958. The biochemistry of organotin compounds. *Biochem. J.* 69:367–376.

Aldridge, W. N. 1976. The influence of organotin compounds on mitochondrial functions. In *Organotin Compounds: New Chemistry and Applications*, ed. J. J. Zuckerman, pp. 186–196. *Advances in Chemistry Series* 157, Washington, DC: American Chemical Society.

Aldridge, W. N., J. E. Casida, R. H. Fish, E. C. Kimmel, and B. W. Street. 1977. Action on mitochondria and toxicity of metabolites of tri-n-butyltin derivatives. *Biochem. Pharmacol.* 26:1997–2000.

Alzieu, C. 2000. Environmental impact of TBT: the French experience. *Sci. Total Environ.* 258:99–102.

Antizar-Ladislao, B. 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environ. Int.* 34:292–308.

Arnold, C. G., A. Weidenhaupt, M. M. David, S. R. Müller, S. B. Haderlein, and R. P. Schwarzenbach. 1997. Aqueous speciation and 1-octanol-water partitioning of tributyl- and triphenyltin: effect of pH and ion composition. *Environ. Sci. Technol.* 31:2596–2602.

Barnes, J. M., and H. B. Stoner. 1958. Toxic properties of some dialkyl and trialkyl tin salts. *Br. J. Ind. Med.* 15:15–22.

Barreiro, B., R. Gonzalez, M. Quintela, and J. M. Ruiz. 2001. Imposex, organotin bioaccumulation and sterility of female *Nassarius reticulatus* in polluted areas of NW Spain. *Mar. Ecol. Prog. Ser.* 218:203–212.

Bartlett, A. J., U. Borgmann, D. G. Dixon, S. P. Batchelor, and R. J. Maguire. 2004. Accumulation of tributyltin in *Hyalella azteca* as an indicator of chronic toxicity: survival, growth, and reproduction. *Environ. Toxicol. Chem.* 23:2878–2888.

Bech, M., J. Strand, and J. A. Jacobsen. 2002. Development of imposex and accumulation of butyltin in the tropical muricid *Thais distinguenda* transplanted to a TBT contaminated site. *Environ. Pollut.* 119:253–260.

Birchenough, A. C., N. Barnes, S. M. Evans, H. Hinz, I. Kronke, and C. Moss. 2002. A review and assessment of tributyltin contamination in the North Sea, based on surveys of butyltin tissue burdens and imposex/intersex in four species of neogastropods. *Mar. Pollut. Bull.* 44:534–543.

Borgert, C. J, T. F. Quill, L. S. McCarty, and A. M. Mason. 2004. Can mode of action predict mixtures toxicity for risk assessment? *Toxicol. Appl. Pharm.* 201:85–96.

Bouchard, N., E. Pelletier, and M. Fournier. 1999. Effects of butyltin compounds on phagocytic activity of hemocytes from three marine bivalves. *Environ. Toxicol. Chem.* 18:519–522.

Brändli, R. C., G. D. Breedveld, and G. Cornelissen. 2009. Tributyltin sorption to marine sedimentary black carbon and to amended activated carbon. *Environ. Toxicol. Chem.* 28:503–508.

Bryan, G. W., P. E. Gibbs, and G. R. Burt. 1988. Comparison of the effectiveness of tri-n-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dog-whelk *Nucella lapillus*. *J. Mar. Biol. Assoc. UK* 68:733–744.

Brüschweiler, B. J, F. E. Würgler, and K. Fent. 1995. Cytotoxicity in vitro of organotin compounds to fish hepatoma cells PLHC-1 (*Poeciliopsis lucida*). *Aquat. Toxicol.* 32:143–160.

Calabrese, E. J., and L. A. Baldwin. 2003. Hormesis: the dose-response revolution. *Annu. Rev. Pharmacol.* 43:175–197.

Cardwell, R. D., and J. P. Meador. 1989. Tributyltin in the environment: an overview and key issues. In *Proceedings of the Organotin Symposium, Oceans 89*. September 18–21, 1989, Seattle, WA, Vol 2: 537–544.

Chagot, D., C. Alzieu, J. Sanjuan, and H. Grizel. 1990. Sublethal and histopathological effects of trace levels of tributyltin fluoride on adult oysters *Crassostrea gigas*. *Aquat. Living Resour.* 3:121–130.

Chow, S. C., et al. 1992. Tributyltin increases cytosolic free Ca^{2+} concentration in thymocytes by mobilizing intracellular Ca^{2+}, activating a Ca^{2+} entry pathway, and inhibiting Ca^{2+} efflux. *Arch. Biochem. Biophys.* 298:143–151.

Ciesielski, T., A. Wasik, I. Kuklik, K. Skora, J. Namiesnik, and P. Szefer. 2004. Organotin compounds in the liver tissue of marine mammals from the Polish Coast of the Baltic Sea. *Environ. Sci. Technol.* 38:1415–1420.

Cima, F., P. J. Craig, and C. Harrington. 2003. Organotin compounds in the environment. In *Organometallic Compounds in the Environment*, ed. P. J. Craig, pp. 101–149. West Sussex: John Wiley and Sons.

Clark, E. A., and R. N. Sterrit. 1988. The fate of tributyltin in the aquatic environment. *Environ. Sci. Technol.* 22:600–603.

Coelho, M. R., W. J. Langston, and M. J. Bebianno. 2006. Effect of TBT on *Ruditapes decussatus* juveniles. *Chemosphere* 63:1499–1505.

Connell, D. W. 1990. Bioconcentration of lipophilic and hydrophobic compounds by aquatic organisms. In *Bioaccumulation of Xenobiotic Compounds*, ed. Connell, D. W., pp. 97–144. Boca Raton, FL: CRC Press.

Connerton, I. F., and D. E. Griffiths. 1989. Organotin compounds as energy-potentiated uncouplers of rat liver mitochondria. *Appl. Organomet. Chem.* 3:545–551.

CRC. 1975. *Handbook of Chemistry and Physics, 56th edition*, ed. R. C. Weast. Cleveland, OH: CRC Press.

de Mora, S. J., N. G. King, and M. C. Miller. 1989. Tributyltin and total tin in marine sediments: profiles and the apparent rate of TBT degradation. *Environ. Technol. Lett.* 10:901–908.

De Santiago, A., and M. Aguilar-Santelises. 1999. Organotin compounds decrease in vitro survival, proliferation and differentiation of normal human B lymphocytes. *Hum. Exp. Toxicol.* 18:619–664.

Di Toro, D. M., J. A. McGrath, and D. J. Hansen. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. water and tissue. *Environ. Toxicol. Chem.* 19:1951–1970.

Dowson, P. H., J. M. Bubb, and J. N. Lester. 1996. Persistence and degradation pathways of tributyltin in freshwater and estuarine sediments. *Estuar. Coast. Shelf Sci.* 42:551–562.

Fent, K. 1996. Ecotoxicology of organotin compounds. *Crit. Rev. Toxicol.* 26:1–117.

Forsyth, D. S., and C. Clerous. 1991. Determination of butyltin, methyltin, and tetraalkyltin in marine food products with gas chromatography-atomic absorption spectrometry. *Talanta* 38:951–957.

Gomez-Ariza, J. L., E. Morales, and I. Giraldez. 1999. Uptake and elimination of tributyltin in clams, *Venerupis decussata*. *Mar. Environ. Res.* 47:399–413.

Grün, F., and B. Blumberg. 2007. Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. *Rev. Endocr. Metab. Dis.* 8:161–171.

Grün, F., H. Watanabe, and Z. Zamanian. 2006. Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. *Mol. Endocrinol.* 20:2141–2155.

Hadjispyrou, S., A. Kungolos, and A. Anagnostopoulos. 2001. Toxicity, bioaccumulation, and interactive effects of organotin, cadmium, and chromium on *Artemia franciscana*. *Ecotox. Environ. Safe.* 49:179–186.

Hagger, J. A., M. H. Depledge, J. Oehlmann, S. Jobling, and T. S. Galloway. 2006. Is there a causal association between genotoxicity and the imposex effect? *Environ. Health Persp.* 114:20–26.

Hano, T., et al. 2007. Tributyltin causes abnormal development in embryos of medaka, *Oryzias latipes*. *Chemosphere* 69:927–933.

Harino, H., M. Fukushima, and S. Kawai. 2000. Accumulation of butyltin and phenyltin compounds in various fish species. *Arch. Environ. Contam. Toxicol.* 39:13–19.

Harino, H., M. Ohji, R. L. Brownell, T. Arai, and N. Miyazaki. 2008. Concentrations of organotin compounds in the stranded killer whales from Rausu, Hokkaido, Japan. *Arch. Environ. Contam. Toxicol.* 55:137–142.

Hoch, M., and D. Schwesig. 2004. Parameters controlling the partitioning of tributyltin (TBT) in aquatic systems. *Appl. Geochem.* 19:323–334.

Horiguchi, T. 1993. Imposex induced by organotin compounds in gastropods in Japan. PhD Dissertation, University of Tokyo. (in Japanese).

Horiguchi, T., H. Shiraishi, M. Shimizu, and M. Morita. 1994. Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. *J. Mar. Biol. Assoc. UK* 74:651–669.

Horiguchi, T., H. Shiraishi, M. Shimizu, and M. Morita. 1997a. Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*. *Environ. Pollut.* 95:85–91.

Horiguchi, T., H. Shiraishi, M. Shimizu, and M. Morita. 1997b. Imposex in sea snails, caused by organotin (tributyltin and triphenyltin) pollutions in Japan: a survey. *Appl. Organomet. Chem.* 11:451–455.

Horiguchi, T., et al. 2002. Tributyltin and triphenyltin induce spermatogenesis in ovary of female abalone. *Haliotis gigantea*. *Mar. Environ. Res.* 54:679–684.

Hu, J., et al. 2006. Trophic magnification of triphenyltin in a marine food web of Bohai Bay, North China: comparison to tributyltin. *Environ. Sci. Technol.* 40:3142–3147.

Hu, J., et al. 2009. Malformations of the endangered Chinese sturgeon, *Acipenser sinensis*, and its causal agent. *P. Nat. Acad. Sci.* 106:9339–9344. plus supporting information.

Huggett, R. J., M. A. Unger, P. F. Seligman, and A. O. Valkirs. 1992. The marine biocide tributyltin. *Environ. Sci. Technol.* 25:232–237.

Hunziker, R. W., B. I. Escher, and R. P. Schwarzenbach. 2002. Acute toxicity of triorganotin compounds: different specific effects on the energy metabolism and role of pH. *Environ. Toxicol. Chem.* 21:1191–1197.

Hwang, H. M., J. R. Oh, S-H. Kahng, and K. W. Lee. 1999. Tributyltin compounds in mussels, oysters and sediments of Chinhae Bay. Korea. *Mar. Environ. Res.* 47:61–70.

IMO. 2001. International Maritime Organization. International Convention on the Control of Harmful Antifouling Systems on Ships. http://www.imo.org (accessed April 20, 2009).

Johansen, K., and F. Mohlenberg. 1987. Impairment of egg production in *Acartia tonsa* exposed to tributyltin oxide. *Ophelia* 27:137–141.

Jones-Lepp, T. L., K. E. Varner, and D. Heggem. 2004. Monitoring dibutyltin and triphenyltin in fresh waters and fish in the United States using micro-lipid chromatography-electrospray/ion trap mass spectrometry. *Arch. Environ. Contam. Toxicol.* 46:90–95.

Kajiwara, N., T. Kunisue, S. Kamikawa, Y. Ochi, S. Yano, and S. Tanabe. 2006. Organohalogen and organotin compounds in killer whales mass-stranded in the Shiretoko Peninsula, Hokkaido, Japan. *Mar. Pollut. Bull.* 52:1066–1076.

Kimbrough, R. D. 1976. Toxicity and health effects of selected organotin compounds: a review. *Environ. Health Persp.* 14:51–56.

King, N., M. Miller, and S. de Mora. 1989. Tributyl tin levels for seawater, sediment, and selected species in coastal Northland and Auckland, New Zealand. *N. Z. J. Mar. Fresh.* 23:287–294.

Kono, K., T. Minami, H. Yamada, H. Tanaka, and J. Koyama. 2008. Bioaccumulation of tributyltin and triphenyltin compounds through the food web in deep offshore water. *Coast. Mar. Sci.* 32:102–107.

Krone, C. A., J. E. Stein, and U. Varanasi. 1996. Butyltin contamination of sediments and benthic fish from the East, Gulf and Pacific coasts of the United States. *Environ. Monit. Assess.* 40:75–89.

Lassiter, R. R., and T. G. Hallam. 1990. Survival of the fattest: implications for acute effects of lipophilic chemicals on aquatic populations. *Environ. Toxicol. Chem.* 9:585–595

Laughlin, R. B. Jr., R. B. Johannesen, W. French, H. Guard, and F. E. Brinckman. 1985. Structure-activity relationships for organotin compounds. *Environ. Toxicol. Chem.* 4:343–351.

Lee, R. F. 1985. Metabolism of tributyltin oxide by crabs, oysters, and fish. *Mar. Environ. Res.* 17:145–148.

Livingstone, D. R. 1998. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comp. Biochem. Physiol.* 120A:43–49.

Lytle, T. F., et al. 2003. Life-cycle toxicity of dibutyltin to the sheepshead minnow (*Cyprinodon variegatus*) and implications of the ubiquitous tributyltin impurity in test material. *Appl. Organomet. Chem.* 17:653–661.

Maguire, R. J. 2000. Review of the persistence, bioaccumulation and toxicity of tributyltin in aquatic environments in relation to Canada's toxic substances management policy. *Water Qual. Res. J. Can.* 35:633–679.

Maguire, R. J., P. T. S. Wong, and J. S. Rhamey. 1984. Accumulation and metabolism of tri-*n*-butyltin cation by green algae, *Ankistrodesmus falcatus*. *Can. J. Fish. Aquat. Sci.* 41:537–540.

Matthiessen, P., and P. E. Gibbs. 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. *Environ. Toxicol. Chem.* 17:37–43.

McAllister, B. G., and D. E. Kime. 2003. Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish (*Danio rerio*). *Aquat. Toxicol.* 65:309–316.

McCarty, L. S. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. *Environ. Toxicol. Chem.* 5:1071–1080.

Meador, J. P. 1986. An analysis of photobehavior in *Daphnia magna* exposed to tributyltin. *Oceans 86 Conference, Organotin Symposium*, Vol. 4:1213–1218. Washington DC, Marine Technological Society.

Meador, J. P. 1993. The effect of laboratory holding on the toxicity response of marine infaunal amphipods to cadmium and tributyltin. *J. Exp. Mar. Biol. Ecol.* 174:227–242.

Meador, J. P. 1997. Comparative toxicokinetics of tributyltin in five marine species and its utility in predicting bioaccumulation and toxicity. *Aquat. Toxicol.* 37:307–326.

Meador, J. P. 2000. Predicting the fate and effects of tributyltin in marine systems. *Rev. Environ. Contam. Toxicol.* 166:1–48.

Meador, J. P. 2006. Rationale and procedures for using the tissue-residue approach for toxicity assessment and determination of tissue, water, and sediment quality guidelines for aquatic organisms. *Hum. Ecol. Risk Assess.* 12:1018–1073.

Meador, J. P., T. K. Collier, and J. E. Stein. 2002. Determination of a tissue and sediment threshold for tributyltin to protect prey species for juvenile salmonids listed by the U.S. Endangered Species Act. *Aquat. Conserv.: Mar. Freshwat. Ecosyst.* 12:539–551.

Meador, J. P., L. S. McCarty, B. I. Escher, and W. J. Adams. 2008. The tissue-residue approach for toxicity assessment: concepts, issues, application, and recommendations. *J. Environ. Monitor.* 10:1486–1498.

Meador, J. P., F. C. Sommers, K. Cooper, G. Yanagida. 2011. Tributyltin and the obesogen metabolic syndrome in a salmonid. *Env. Res.* In press.

Mercier, A., E. Pelletier, and J-F. Hamel. 1994. Metabolism and subtle toxic effects of butyltin compounds in starfish. *Aquat. Toxicol.* 28:259–273.

Morcillo, Y., and C. Porte. 1999. Evidence of endocrine disruption in the imposex-affected gastropod *Bolinus brandaris*. *Environ. Res.* 81:349–354.

Nagase, H., T. Hamasaki, T. Sato, H. Kito, Y. Yoshioka, and Y. Ose. 1991. Structure-activity relationships for organotin compounds on the red killifish *Oryzias latipes*. *Appl. Organomet. Chem.* 5:91–97.

Nakayama, K., Y. Oshima, K. Nagafuchi, T. Hano, Y. Shimasaki, and T. Honjo. 2005. Early-life stage toxicity in offspring from exposed parent Medaka, *Oryzias latipes*, to mixtures of tributyltin and polychlorinated biphenyls. *Environ. Toxicol. Chem.* 24:591–596.

Nakayama, K., Y. Oshima, and T. Yamaguchi. 2004. Fertilization success and sexual behavior in male Medaka, *Oryzias latipes*, exposed to tributyltin. *Chemosphere* 55:1331–1337.

Nishikawa, J-I., et al. 2004. Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods. *Environ. Sci. Technol.* 38:6271–6276.

Oehlmann, J., P. Fioroni, E. Stroben, and B. Markert. 1996. Tributyltin (TBT) effects on *Ocinebrina aciculata* (Gastropoda: Muricidae): imposex development, sterilization, sex change and population decline. *Sci. Total Environ.* 188:205–223.

O'Halloran, K., J. T. Ahokas, and P. F. A. Wright. 1998. Response of fish immune cells to in vitro organotin exposures. *Aquat. Toxicol.* 40:141–156.

Ohji, M., T. Arai, and N. Miyazaki. 2006. Transfer of tributyltin from parental female offspring in the viviparous surfperch (*Ditrema temmincki*). *Mar. Ecol. Prog. Ser.* 307:307–310.

Rehage, J. S., S. G. Lynn, J. I. Hammond, B. D. Palmer, and A. Sih. 2002. Effects of larval exposure to triphenyltin on the survival, growth, and behavior of larval and juvenile *Ambystoma barbouri* salamanders. *Environ. Toxicol. Chem.* 21:807–815.

Rüdel, H., P. Lepper, and J. Steinhanses. 2003. Retrospective monitoring of organotin compounds in marine biota from 1985 to 1999: results from the German Environmental Specimen Bank. *Environ. Sci. Technol.* 37:1731–1738.

Saarikoski, J., and M. Viluksela. 1982. Relation between physiochemical properties of phenols and their toxicity and accumulation in fish. *Ecotox. Environ. Safe.* 6:501–512.

Salazar, M. H., and S. M. Salazar. 1995. In-situ bioassays using transplanted mussels: I. Estimating chemical exposure and bioeffects with bioaccumulation and growth. In *Environmental Toxicology and Risk Assessment, Third Volume*, eds. G. R. Biddinger, E. Mones, and J. S. Hughes, pp. 216–241. Philadelphia, PA: American Society for Testing and Materials.

Salazar, M. H., and S. M. Salazar. 1998. Using caged bivalves as part of an exposure-dose-response triad to support an integrated risk assessment strategy. In *Proceedings–Ecological Risk Assessment: A meeting of Policy and Science*, eds. A. de Peyster and K. Day, pp. 167–192. Pensacola, FL: SETAC Press.

Schulte-Oehlmann, U., J. Oehlmann, B. Bauer, P. Fioroni, and U-S. Leffler. 1998. Toxico-kinetic and -dynamic aspects of TBT-induced imposex in *Hydrobia ulvae* compared with intersex in *Littorina littorea* (Gastropoda, Prosobranchia). *Hydrobiologia* 378:215–225.

Seligman, P. F., A. O. Valkirs, P. M. Stang, and R. F. Lee. 1988. Evidence for rapid degradation of tributyltin in a marina. *Mar. Pollut. Bull.* 19:531–534.

Seligman, P. F., et al. 1989. Distribution and fate of tributyltin in the United States marine environment. *Appl. Organomet. Chem.* 3:31–47.

Shim, W. J., et al. 2005. Accumulation of butyl- and phenyltin compounds in starfish and bivalves from the coastal environment of Korea. *Environ. Pollut.* 133:489–499.

Shimasaki, Y., T. Kitano, Y. Oshima, S. Inoue, N. Imada, and T. Honjo. 2003. Tributyltin causes masculinization in fish. *Environ. Toxicol. Chem.* 22:141–144.

Shimasaki, Y., et al. 2006. Effect of tributyltin on reproduction in Japanese whiting, *Sillago japonica*. *Mar. Environ. Res.* 62:S245–S248.

Stäb, J. A., T. P. Traas, G. Stroomberg, J. van Kesteren, P. Leonards, and B. van Hattum. 1996. Determination of organotin compounds in the foodweb of a shallow freshwater lake in the Netherlands. *Arch. Environ. Contam. Toxicol.* 31:319–328.

Stoner, H. B. 1966. Toxicity of triphenyltin. *Br. J. Ind. Med.* 23:222–229.

Takahashi, S., S. Tanabe, I. Takeuchi, and N. Miyazaki. 1999. Distribution and specific bioaccumulation of butyltin compounds in a marine ecosystem. *Arch. Environ. Contam. Toxicol.* 37:50–61.

Tanabe, S. 1999. Butyltin contamination in marine mammals—a review. *Mar. Pollut. Bull.* 39:62–72.

Tang, C-H., and W-H. Wang. 2008. Butyltin accumulation in two marine bivalves along a pollution gradient. *Environ. Toxicol. Chem.* 27:2179–2185.

Tas, J. W. 1993. Fate and effects of triorganotins in the aqueous environment: bioconcentration kinetics, lethal body burdens, sorption and physico-chemical properties. PhD Dissertation, University of Utrecht, The Netherlands, 205 pp.

Tessier, E., et al. 2007. (Tri)butyltin biotic degradation rates and pathways in different compartments of a freshwater model ecosystem. *Sci. Total Environ.* 388:214–233.

Tolosa, I., L. Merlini, N. De Bertrand, J. M. Bayona, and J. Albaiges. 1992. Occurrence and fate of tributyl- and triphenyltin compounds in western Mediterranean coastal enclosures. *Environ. Toxicol. Chem.* 11:145–155.

Triebskorn, R., H-R. Kohler, J. Flemming, T. Braunbeck, R-D. Negele, and H. Rahmann. 1994. Evaluation of bis(tri-n-butyltin)oxide (TBTO) neurotoxicity in rainbow trout (*Oncorhynchus mykiss*). 1. Behaviour, weight increase, and tin content. *Aquat. Toxicol.* 30:189–197.

Tsuda, T., S. Aoki, M. Kojima, and T. Fujita. 1992. Accumulation and excretion of tri-n-butyltin chloride and triphenyltin chloride by willow shiner. *Comp. Biochem. Physiol.* 101C:67–70.

Tsuda, T., S. Aoki, M. Kojima, and H. Harada. 1990. The influence of pH on the accumulation of tri-n-butyltin chloride and triphenyltin chloride in carp. *Comp. Biochem. Physiol.* 95C:151–153.

Tsuda, T., S. Aoki, M. Kojima, and H. Harada. 1991. Accumulation of tri-n-butyltin chloride and triphenyltin chloride by oral and via gill intake of goldfish (*Carassius auratus*). *Comp. Biochem. Physiol.* 99C:69–72.

Tsuda, T., M. Wada, S. Aoki, and Y. Matsui. 1988. Bioconcentration, excretion and metabolism of bis(tri-*n*-butyltin) oxide and triphenyltin chloride by goldfish. *Toxicol. Environ. Chem.* 18:11–20.

U.S. Congress. 1988. Organotin antifouling paint control act of 1988. Public Law 100-333 (33 USC 2401). 100th Congress of the United States of America, Second Session. Washington, DC.

U.S. EPA. 1997. Toxicological Review. Tributyltin. Integrated Risk Information System (IRIS), Washington, DC.

U.S. EPA. 2003. Ambient Aquatic Life Water Quality Criteria for Tributyltin (TBT)—Final. EPA 822-R-03-031. Environmental Protection Agency, Office of Water, Washington DC.

Veltman, K., M. A. J. Huijbregts, M. J. van den Heuvel-Greve, A. D. Vethaak, and A. J. Hendriks. 2006. Organotin accumulation in an estuarine food chain: comparing field measurements with model estimations. *Mar. Environ. Res.* 61:511–530.

Vighi, M., and D. Calamari. 1985. QSARs for organotin compounds on *Daphnia magna*. *Chemosphere* 14:1925–1932.

Walsh, T. J., and D. L. DeHaven. 1988. Neurotoxicity of the alkyltins. In *Metal Neurotoxicity*, eds. S. C. Bondy, and K. N. Prasad, pp. 87–107. Boca Raton, FL: CRC Press.

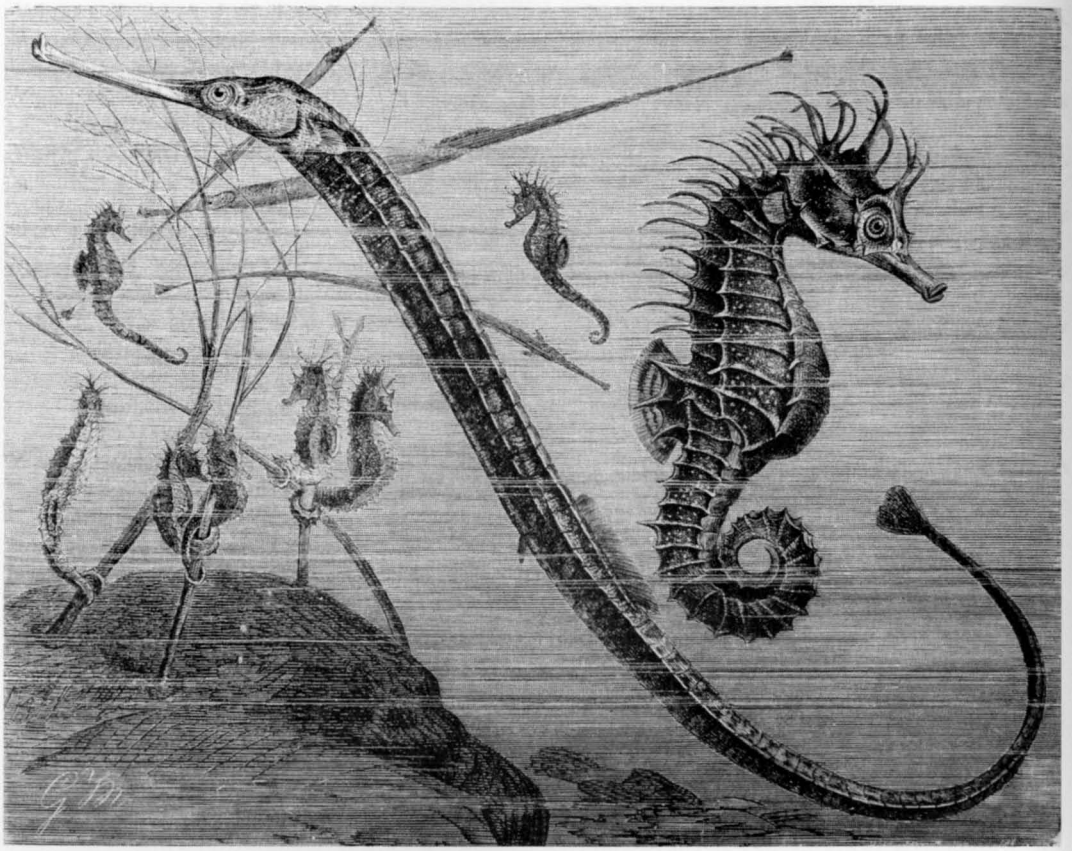
Widdows, J., and D. S. Page. 1993. Effects of tributyltin and dibutyltin on the physiological energetics of the mussel *Mytilus edulis*. *Mar. Environ. Res.* 35:233–249.

Wong, P. T. S., Y. K. Chau, O. Kramar, and G. A. Bengert. 1982. Structure-activity relationship of tin compounds on algae. *Can. J. Fish. Aquat. Sci.* 39:483–488.

Yang, J., and N. Miyazaki. 2006. Transplacental transfer of butyltins to fetus of Dall's porpoises (*Phocoenoides dalli*). *Chemosphere* 63:716–721.

Zhang, Z., J. Hu, H. Zhen, X. Wu, and C. Huang. 2008. Reproductive inhibition and transgenerational toxicity of triphenyltin on medaka (*Oryzias latipes*) at environmentally relevant levels. *Environ. Sci. Technol.* 42:8133–8139.

Zou, Z., J. Cai, X. Wang, B. Li, C. Wang, and Y. Chen. 2009. Acute administrations of tributyltin and trimethyltin modulated glutamate and N-methyl-D-aspartate receptor signaling pathway in *Sebastiscus marmoratus*. *Aquat. Toxicol.* 92:44–49.



Great Pipe-Fish and Short-Snouted Sea-Horse

By G. Mutzel, from *The Royal Natural History*, edited by Richard Lydekker, Frederick Warne & Co., London, 1893–94.