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Organotins in Aquatic Biota: Occurrence in Tissue and Toxicological Significance

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James P. Meador

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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

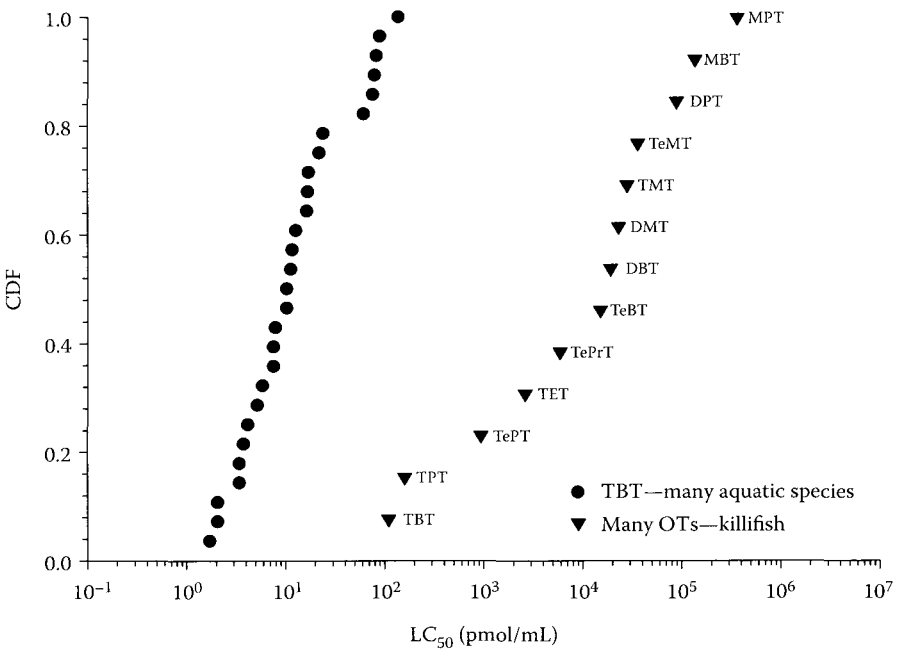

FIGURE 7.1 Circles show 96 h LC_{50} values in pmol/mL for 28 aquatic species (polychaetes, amphipods, copepods, shrimp, and fish) exposed to TBT in water. Data from Cardwell and Meador (1989). Triangles are the 48 h LC_{50} values for one species (killifish, *Oryzias latipes*) exposed to many different 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

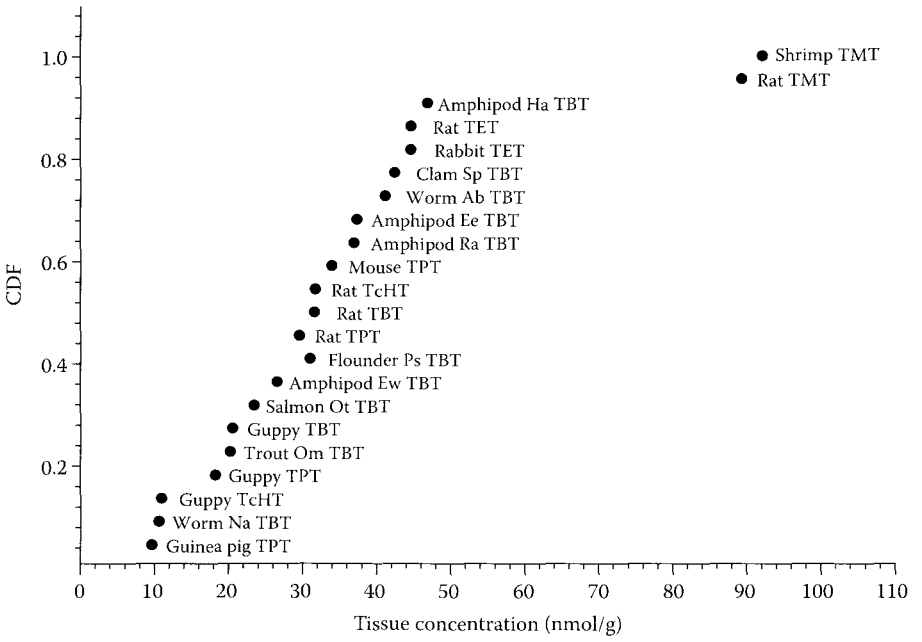

FIGURE 7.2 Values are whole-body lethal concentrations (LR_{50}) for 15 species and five organotins. The small mammal values are equivalent to the intraperitoneal injection concentration. Values range from 10.6 to 100 nmol/g wet weight and species names are centered on the value (e.g., guinea pig TPT 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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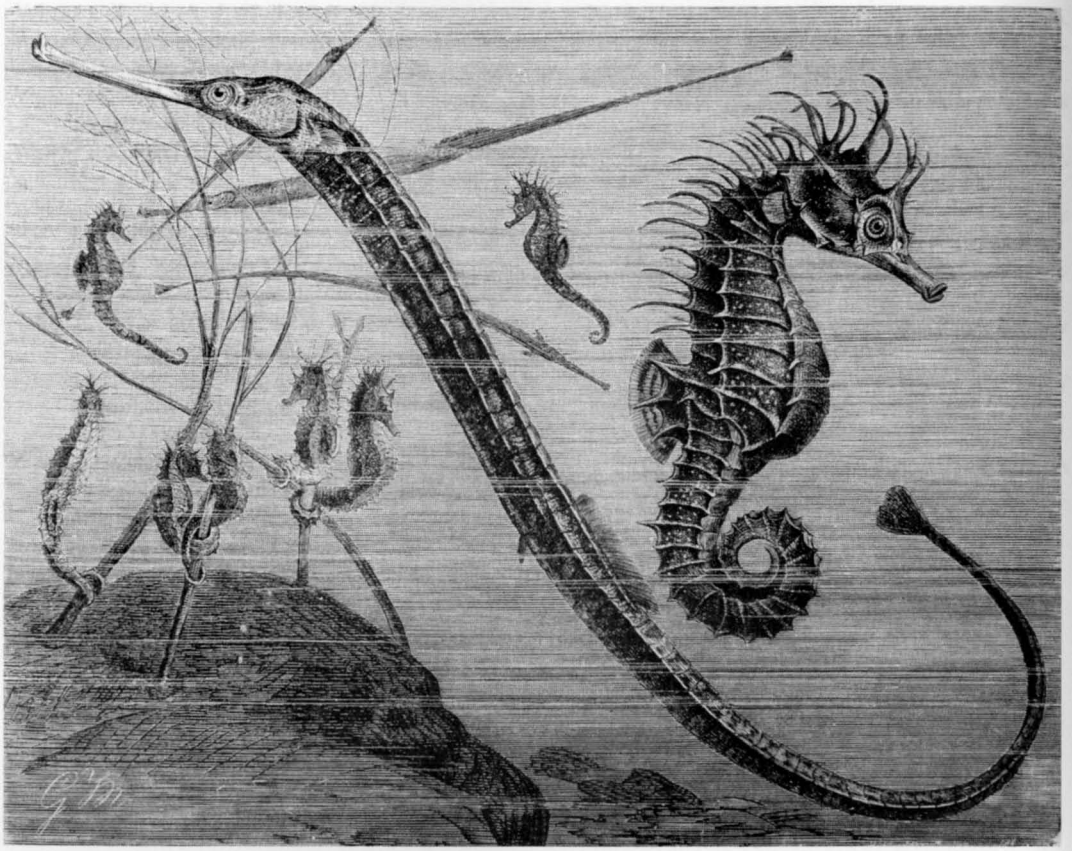

Great Pipe-Fish and Short-Snouted Sea-Horse

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