

Chapter 16

Molluscs

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16.1 Introduction

Concern over organotin (OT) bioaccumulation has focused on molluscs due to the effects of TBT and TPT on reproduction and recruitment in this phylum at extremely low concentrations. There have also been concerns because molluscs form an important component of food chains involving humans. Molluscs represent a significant, if variable, concentration step in the transfer of OTs from water (Bioconcentration Factor-BCF $\sim 10^2$ – 10^5) and sediment (BCF_{sed} up to 10^2) – an attribute which has been harnessed in biomonitoring programmes. Few phyla display comparable abilities for bioconcentration of OTs, which accounts for their sensitivity. However, bioaccumulation is not always simply a function of adsorption of dissolved forms (except at the lowest trophic levels), but may also involve uptake from dietary sources including sediments, and modification by metabolism and excretion, giving rise to much variability. In this chapter we review the pathways and potential for bioaccumulation of OTs in three major classes of the Mollusca, namely gastropods, bivalves and cephalopods. Much of the knowledge gained from studies on OTs will have broader implications – in terms of understanding the processes and timescales of impacts of future persistent contaminants, and, hopefully, in the design of future risk assessment protocols.

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16.2 Background – The ‘Special Relationship’ Between TBT and Molluscs

In the late 1970s and early 1980s, stocks of oysters *Crassostrea gigas* near marinas in France and the UK failed, or were unmarketable due to deformation of the shells. TBT was subsequently linked with reduced spatfall and unnatural shell thickening (Waldock and Thain 1983; Alzieu et al. 1986). Shortly after this, TBT was also shown to cause masculinisation (imposex) in female stenoglossan gastropods *Nucella lapillus*, with widespread population decline observed near to marinas and ports (Bryan et al. 1986). The sensitivity of both shell thickening and imposex responses led to their use as indicators of OT pollution world-wide. Recognition of effects in molluscs resulted in many nations enforcing partial legislation by the early 1990s. Since then it has been shown that numerous non-target species may be adversely affected by OTs, though it is usually mollusc species which are among the most sensitive.

Prior to legislation, elevated concentrations of TBT in spring and summer (linked to increased boating activity) coincided with the onset of the breeding season of many molluscs, exacerbating the threat. However it was not only pulses of TBT in water that posed hazards. TBT preferentially adsorbs on to phytoplankton and suspended particulates ($K_d \sim 10^4$ in fine silts) which constitute the diet of filter-feeders. If deposited in benthic sediments, these particulate loadings can persist, particularly in anoxic conditions (Chapter 5). Organotin-laden paint flakes may also be entrained in sediments near dockyards and marinas. If only a fraction of this particulate material is biologically available, consequences for sediment-dwelling molluscs become important and can be severe. Additionally, the carnivorous diet of cephalopods and some gastropods adds the potential for food chain transfer.

In the following sections, evidence showing how assimilation and degradation pathways influence bioaccumulation is presented. Molluscs clearly exhibit a large range in behaviour with respect to OT. Triorganotins display maximum bioaccumulation within any homologous series: hence accumulation (and toxicity) of TBT/TPT far exceeds that of DBT/DPT and MBT/MPT. However, few studies in the west report significant data for TPT, reflecting much lower usage compared with Japan (where it is now banned), Korea and some other eastern countries. Consequently, much of this review is based on observations on TBT.

16.3 Gastropods

TBT (and TPT) are most harmful to stenoglossan gastropods, by virtue of effects on reproduction, and at least 150 species are now thought to be susceptible to imposex. Body burdens of only $0.01 \mu\text{g TBT g}^{-1}$ may initiate imposex in the most sensitive species and both dietary and aqueous sources appear equally important for bioaccumulation.

Bioconcentration factors in gastropods exposed to TBT in the laboratory, via water, vary between 10^3 and 10^5 . In *Nucella lima* the BCF was $\sim 4 \times 10^3$ (at 64 ng TBT l^{-1}) and,

Table 16.1 Summary of TBT bioconcentration parameters in molluscs from field studies and laboratory investigations

Mollusc	Exposure ^a	BCF ^b (water)	BCF ^b (sediment)	Steady state (days)	Half time (days)	Notes ^c	Reference
Gastropods							
<i>Nucella lapillus</i>	L	~30,000 at 8 ng l ⁻¹		50–100 ^d	50–60	52–80% TBT	Bryan et al. (1986, 1987)
		~16,000 at 50 ng l ⁻¹					
		~10,000 at 267 ng l ⁻¹				ww (x3 for dw)	
	F(transplant)	~15,000 (~67 ng l ⁻¹)			~100		
<i>Nucella lima</i>	L	4,390 at 64 ng l ⁻¹		~60		22–60% TBT (4 months); ww	Stickle et al. (1990)
		2,050 at 914 ng l ⁻¹					
<i>Littorina littorea</i>	F	4,400	3.9	6 m		35% TBT; ww	Langston et al. (1987)
<i>Littorina littorea</i>	L	5,100–7,100	0.01–0.33			dw	Bauer et al. (1997)
	F	(2–400 ng l ⁻¹) 1,170–34,600				dw	
<i>Littorina littorea</i>	F	500–10,000	0.97			25% TBT	Harino et al. (2005b)
<i>Littorina littorea</i>	F					20–40% TBT winter	Kure and Depledge (1994)
<i>Hinia reticulata</i>	L	11,500 at 9 ng l ⁻¹	0.95	70 ^d	18–26	60–70% TBT spring dw	Pope (1998)
Bivalves							
<i>Crassostrea gigas</i>	F	25,000				Estimated	Shim et al. (1998)
<i>Crassostrea gigas</i>	L	2,000 at 1,250 ng l ⁻¹		14	~10–23	ww	Waldock et al. (1983)
	F	6,000 at 150 ng l ⁻¹					
		10,000					

(continued)

Table 16.1 (continued)

Mollusc	Exposure ^a	BCF ^b (water)	BCF ^b (sediment)	Steady state (days)	Half time (days)	Notes ^c	Reference
<i>Ostrea edulis</i>	L	1,000 at 1,250 ng l ⁻¹ 1,500 at 150 ng l ⁻¹ 2,000		14	~10–23	ww	Waldock et al. (1983)
<i>Aequipecten irradians</i>	L	2,000–10,000		60 ^d		ww	Guolan and Yong (1995)
<i>Mytilus edulis</i>	L	1,813 at 800 ng l ⁻¹ 1–9,000 at 50 ng l ⁻¹		28 ^d	36–21	>74% TBT; dw	Yang et al. (2006)
<i>Mytilus edulis</i>	L	5,000 at 500 ng l ⁻¹		47 ^d	14	ww	Laughlin et al. (1986)
<i>Mytilus edulis</i>	L	7,700–11,000		60 ^d		ww	Guolan and Yong (1995)
<i>Mytilus edulis</i>	F(transplant)	5,000–60,000		51 ^d	40	ww (x~8 for dw)	Zuolian and Jensen (1989)
<i>Mytilus edulis</i>	F		0.55			28% TBT	Harino et al. (2005b)
<i>Dreissena polymorpha</i>	F(transplant)	900,000				>90% TBT; dw	Van Slooten and Tarradellas (1994)
<i>Mya arenaria</i>	F	133,000	77.9			83% TBT; ww	Langston et al. (1987)
<i>Mya arenaria</i>	F	57,000–220,000	25–384			80–90% TBT; dw	Kure and Depledge (1994)
<i>Mya arenaria</i>	L	91,800 at 50 ng l ⁻¹ 44,800 at 200 ng l ⁻¹ 15,538 at 800 ng l ⁻¹		28 ^d	71	>89% TBT; dw	Yang et al. (2006)
<i>Ruditapes decussatus</i>	L	9,000 at 100 ng l ⁻¹	<1	28 ^d	87	>89% TBT; dw	
				20 water	94	>89% TBT; dw	
				40 sed		dw	Coelho et al. (2002a, c)
<i>Nuculana pernula</i>	F		67–461			77–88% TBT	Strand et al. (2003)
<i>Elliptio complanata</i>	F(transplant)	4,800–18,500	0.1–18	140 ^d		Mainly TBT; ww	Chau et al. (1989)

<i>Scrobicularia plana</i>	F	96,000	9	40	50	dw	Langston and Burt (1991)
	L	13,000 at 100 ng l ⁻¹	11		28		
<i>Scrobicularia plana</i>	F		3.1			20% TBT	Harino et al. (2005b)
<i>Cerastoderma edule</i>	F		2.7			38% TBT	Harino et al. (2005b)
<i>Macoma balthica</i>	F		3.9			25% TBT	Harino et al. (2005b)
<i>Macoma nasuta</i>	L	10,400 at 200 ng l ⁻¹		60			Salazar et al. (1987)
Cephalopods							
<i>Todarodes pacificus</i>	F	18,000–101,000 (digestive gland)				ww	Yamada et al. (1997)

^aL = laboratory, F = field.

^bBCF – tissue bioconcentration factor relative to water or sediment.

^cNotes: BCF_{water} results expressed on wet weight (ww) or dry weight (dw) basis; percentages refer to proportion of extractable BT present as TBT.

^dApproaching, but not at, steady state.

although not particularly high in comparison with other molluscs (Table 16.1), resultant body burdens were sufficient to cause imposex (Stickle et al. 1990). TBT was also bioaccumulated from food (naturally contaminated mussels) in comparable amounts, though biomagnification of TBT in *N. lima*, relative to food, did not occur.

BCFs in the dog whelk *Nucella lapillus* decrease with increasing contamination, implying dose-dependent tissue saturation; nevertheless at ~50–70 ng TBT l⁻¹ the BCF was estimated to be ~16,000 (Bryan et al. 1986, 1987) – higher than in *N. lima* and perhaps explaining the greater sensitivity of *N. lapillus*. Steady state is approached after 50 days aqueous exposure in *N. lapillus* but further accumulation can take place in females, notably during egg development. It is possible that TBT metabolism is more efficient in *N. lima*. However, degradation/elimination of TBT in *N. lapillus* increases (and BCFs decline) with increasing contamination, such that only half the total BT is present as TBT at high doses. Elimination half-times for TBT in *N. lapillus* range from 50 to 60 days, but may vary depending on physiological condition and may be as long as 100 days in the field, perhaps signifying the presence of pools with different rates of exchange (Bryan et al. 1987).

Nucella lapillus fed on ¹⁴C-TBT labelled *Mytilus edulis* displayed assimilation efficiencies approaching 100%. When body burdens were compared with dogwhelks exposed via water only, it was estimated that up to half the body burden of TBT could originate from the diet, except perhaps in winter, when feeding rate declines (Bryan et al. 1989). Nevertheless, food chain biomagnification of TBT does not occur because metabolism of TBT appears to be more efficient in dogwhelks than in mussels – only 65% of measurable butyltins was present as the parent compound in *Nucella*, compared with 90% TBT in *Mytilus* (Bryan et al. 1989, 1993a). TBT tissue distributions were indicative of uptake routes and *N. lapillus* fed on TBT-labelled mussels absorbed dietary TBT most efficiently via the digestive gland (where it was also degraded); in contrast absorption of dissolved TBT occurred primarily in the mantle and gills – tissues in direct contact with the water. Kidney was also important in uptake and degradation in both scenarios. Bryan et al. (1993a) provide a useful conceptual model showing relationships between uptake route, degradation and tissue distribution in these carnivorous gastropods.

In a number of neogastropod species in Japan, degrees of imposex have been shown to reflect pollution levels, not only of TBT, but also TPT (Horiguchi et al. 1994). Estimated body burdens of TBT responsible for inducing imposex were 10–20 ng g⁻¹ (wet wt) for rock shells, *Thais clavigera* and *T. bronni* – similar to 'effect levels' for the initiation of imposex in *Nucella* spp.

Littorina littorea, a mesogastropod species, exhibits a form of imposex (intersex) but is much less sensitive to TBT than neogastropods – body burdens required to initiate the condition are at least an order of magnitude higher. Nevertheless, these concentrations, and accompanying symptoms, occur near marinas and harbours. The severity of intersex increases as a function of body burden which, in turn, increases in relation to both water and sediment contamination (Bauer et al. 1997). *L. littorea* exhibit rather low BCFs, especially where contamination is high (Table 16.1) and this could contribute to their lower sensitivity. Possible routes of organotin uptake in *Littorina*, besides water, could include the diet of macroalgae (generally low in TBT) and sediment (unlikely, since BCF_{sed} are usually <1),

though neither pathway has been quantified. Also, degradation of TBT appears to be rapid in this species, as reflected in seasonal changes in BTs: prior to legislation, approximately 60–70% of the total organotin was present as TBT in spring (presumably because of new inputs from paint) but this proportion fell to 20–40% during autumn and winter, implying effective metabolism.

In the burrowing neogastropod *Hinia reticulata*, BCF factors of 28,000–74,500 have been reported for TBT at seawater concentrations in the range 2.5–47 ng l⁻¹ (Stroben et al. 1992; Bryan et al. 1993), though both field studies acknowledge that these BCF values may have been overestimates, due to contributions from other sources (diet). Experiments with ¹⁴C-TBT have confirmed *H. reticulata* to be an effective accumulator of TBT from seawater with concentration factors of 10³–10⁴ over a 50 day exposure period (Pope 1998; Table 16.1). However, these studies also revealed that much of the TBT body burden was accumulated from sediments (in equilibrated systems) and that this sediment-derived proportion increased as the sediment TBT concentration increased (31% at 67 ng g⁻¹ TBT, 87% at 620 ng g⁻¹ TBT). The mechanism whereby *H. reticulata* accumulates TBT from sediment is primarily by direct contact, involving adsorption across the respiratory surfaces and also the exposed tissue of its head/foot – a large flat area of tissue which is in direct contact with the sediment and pore water as the animal burrows. Ingestion of sediment is not considered a major uptake route, since bioaccumulation in individuals with a ligatured proboscis is comparable to that in ‘normal’ snails. In the wild, however, incidental ingestion of some sediment during feeding (on dead biota) cannot be discounted (Pope 1998).

Metabolism of TBT in *Hinia* occurs predominantly in the digestive gland and the more polar debutylated products are excreted via the kidney. Half-times for butyltin depuration, determined following injection of ¹⁴C-TBT, were fairly short – between 26 and 18 days (Pope 1998). Within this depuration period, butyltin speciation changed significantly – from >70% as TBT during the first week of depuration to <29% TBT after 90 days. The corresponding DBT proportion increased from <10% to >50% over the same period, while the fraction as MBT remained relatively constant at around 20%. Following exposure via sediment and water, half-times were somewhat longer, ranging from 30 to 66 days.

16.4 Bivalves

This class of mollusc encompasses a variety of filter-feeding types including suspension feeders (oysters, mussels and some clams) and more specialized deposit-feeding clams, giving rise to diverse OT behaviour.

Oysters have been the subject of a number of bioaccumulative and monitoring studies in view of the deleterious effects of OT demonstrated near marinas and harbours (Waldock and Thain 1983; Alzieu et al. 1986; Shim et al. 1998). Uptake of TBT is fairly rapid in both *Crassostrea gigas* and *Ostrea edulis* which reach steady-state within 14 days during laboratory exposures – at BCFs in the range 10³–10⁴ (decreasing with increasing contamination). Bioaccumulation capacity is higher

in *C. gigas* than in *O. edulis* (by up to four-fold and nine-fold in laboratory and field, respectively, Table 16.1). Despite initial rapid losses in laboratory-exposed individuals, depuration in *C. gigas* tends to be prolonged in nature, even when sources are reduced, probably due to a slowly exchanging residual pool (Waldock et al. 1983). Loss rates under field conditions are illustrated by the reduction in tin levels in *C. gigas* from Arcachon Bay – from $3.40 \mu\text{g g}^{-1}$ dry wt in 1982 (the year the French banned the use of TBT-based paints on all vessels under 25 m length) to $0.50 \mu\text{g g}^{-1}$ 2 years later (Alzieu et al. 1986). Fairly slow metabolism of TBT is indicated in laboratory experiments with *Crassostrea virginica*, attributable to low levels of cytochrome P450-mediated transformations (Lee 1986).

Mussels are relatively ubiquitous and TBT accumulation has been shown to be positively related to seawater TBT concentrations, providing time-integrated information on OT contamination. Consequently they too have been used widely as monitoring organisms (Salazar and Salazar 1996; Harino et al. 1998, 1999). Relatively poor correlations between TBT concentrations in mussels (and oysters) and benthic sediments appears to confirm that the water column, including phytoplankton, is the more important source in these suspension feeders (Wade et al. 1990).

As in other molluscs, BCFs in *Mytilus edulis* exposed experimentally to aqueous TBT exhibit an inverse relationship with dose (Table 16.1): for example, at $\sim 50 \text{ ng l}^{-1}$ the BCF was of the order of 9,000 (Yang et al. 2006) compared with 5,000 at 500 ng l^{-1} (Laughlin et al. 1986). Field studies, including transplants, also indicate an inverse (exponential) correlation between BCFs and water concentration (BCF range 5,000–>100,000, but mostly 20–40,000: Zuolian and Jensen 1989; Salazar and Salazar 1996). Extremely high BCFs (900,000) were recorded for the freshwater mussel *Dreissena polymorpha* following transplantation to a TBT-contaminated marina (Van Slooten and Tarradellas 1994).

Loss of OTs from *Mytilus edulis* is fairly rapid during depuration experiments (half life 14–40 days), and is consistent with a first-order kinetic model. However, differences in elimination rate constants have been observed during uptake and release phases, which suggests behaviour in the laboratory and field could be different, contributing to variations in estimated BCF (Laughlin et al. 1986). In nature, higher BCFs could also signify an additional contribution from food. Mussels, like oysters, are epibenthic filter feeders capable of filtration rates of 60 l h^{-1} and selective removal of particles down to a few micrometers diameter (principally phytoplankton, together with microorganisms adhering to suspended particulates). Phytoplankton have been confirmed as an important vector for TBT assimilation in mussels, although there is no indication of TBT biomagnification (transfer factor <2) (Laughlin et al. 1986; Guolan and Yong 1995).

Viscera and gills of mussels are dominant sites for TBT bioaccumulation from diet and water, respectively. Despite a general correlation between TBT accumulation and lipid content of mussel tissues (in terms of ranking), TBT concentrations in mussels are an order of magnitude higher than predicted from solubility models, implying that bioaccumulation is not governed by lipid partitioning behaviour alone, but may be modified by physiology – for example, binding to proteins, including enzymes (Laughlin et al. 1986; Guolan and Yong 1995). Degradation does not appear to differ greatly between different mussel tissues and in the exam-

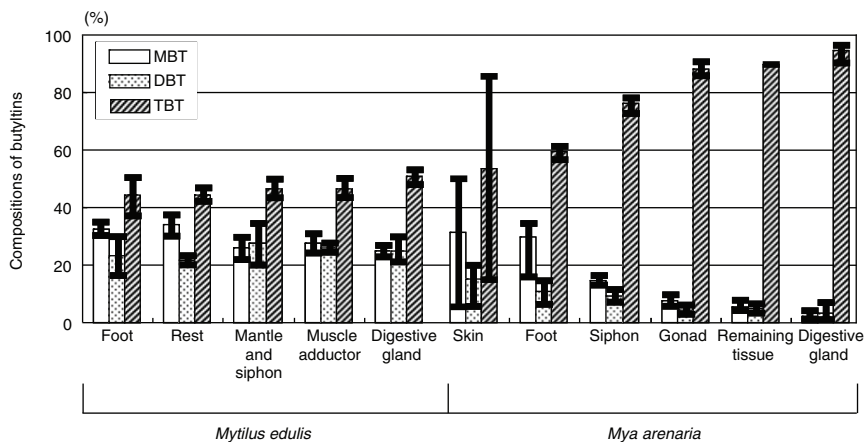


Fig. 16.1 Composition of butyltins in tissues of *Mytilus edulis* and *Mya arenaria*, Mersey Estuary (Reprinted from Harino et al. 2005a. With permission from Elsevier)

ple for *Mytilus edulis* from the Mersey Estuary shown in Fig. 16.1, TBT typically constituted about half the total BT content in most tissues, with the remainder made up equally by DBT and MBT (Harino et al. 2005a).

Infaunal clams in sediments may, in principle, be exposed to additional OT contamination associated with the particulate phase, in addition to that in the water column. The disparity in BCFs between field and laboratory studies often indicates the presence of alternative sources and has prompted several studies on the relative importance of diet (including sediment) on organotin bioaccumulation in clams.

The affinity of TBT for particulates, in particular organic-rich fines ($K_d \sim 10^4$), would be anticipated to be particularly important for deposit-feeding clams. Thus, TBT burdens in the infaunal clam *Scrobicularia plana* collected from estuaries around the UK were found to be directly related to sediment loadings and, in experiments, *S. plana* was shown to accumulate >90% of its TBT burden from this source (Langston and Burt 1991). BCF_{sed} were similar in the field and laboratory, at comparable exposures (Table 16.1), and decreased as sediment TBT loadings increased. The kinetics of TBT uptake from sediment indicated the approach of steady-state in *S. plana* after 40 days. Initial loss of TBT from clams during depuration was fairly rapid (half-time 15–30 days depending on dose), but consisted of two compartments, one of which appeared very persistent. In the field the half-time for loss was longer – of the order of 50 days. Given that TBT is likely to persist for some time in sheltered sediments close to harbours and dockyards, the impact on populations of deposit feeders such as *S. plana* may continue for some time compared with other types of mollusc.

Suspension feeding clams *Ruditapes decussatus* appear less vulnerable to TBT in benthic sediments than *S. plana* since, in equilibrated systems, uptake is predominantly (>90%) from water (Coelho et al. 2002a). Although some uptake from sediment does occur, the processing of large amounts of water needed to sustain the suspension-feeding habit of this species is the primary route of TBT assimilation (accounting for body burdens 30 times higher than from sediment). Field surveil-

lance of TBT contamination using *R. decussatus* indicates significant correlation with water, but not sediment, consistent with laboratory results (Coelho et al. 2002b). Suspended microalgae in the water column are a potential source of OTs in clams such as *R. decussatus*, though in experiments using moderate cell densities of ^{14}C TBT – labelled *Isochrysis galbana*, this route appeared to be overshadowed (100-fold) by direct uptake from the aqueous phase (Coelho et al. 2002c). At steady state (40 days) the transfer factor – TBT *R. decussatus*/TBT *I. galbana* – was 0.3, indicating that food chain biomagnification from phytoplankton to clams was not occurring. However, the relative contribution towards body burdens in nature is somewhat uncertain and will vary according to the quantity and quality of microalgal cells. Where phytoplankton productivity is high, more of the TBT in the water column will be bound to algal cells, representing a larger vector for assimilation. Interestingly, similar BCFs were obtained in *R. decussatus* exposed to either sediments or *Isochrysis*, implying that TBT was assimilated to an equivalent degree from both particulate forms.

Tissue TBT distributions in *Ruditapes decussatus* reflected the major source of uptake—with gills representing the most important site for bioaccumulation from water (Coelho et al. 2002a). The digestive gland accumulated TBT preferentially from food (*Isochrysis galbana*), initially, though after a few weeks of exposure, internal remobilization resulted in a more widespread partitioning of TBT amongst tissues (Coelho et al. 2002c).

The deep-burrowing soft-shell clam *Mya arenaria* is an extraordinary accumulator of BTs, particularly TBT. Butyltins represent a dominant proportion of the total tin in *Mya* (~97%). In contrast, BTs represent ~1% of the total tin in sediment, demonstrating that BTs are considerably more bioavailable than inorganic Sn (the principal particulate form), and are effectively and selectively assimilated. At the height of the TBT problem in the 1980s, BCF in *M. arenaria* from Poole Harbour, UK, were of the order of 133×10^3 on a wet weight basis – or $>500 \times 10^3$ on a dry weight basis – some ten times higher than other bivalves and 30-fold higher than the gastropod *Littorina littorea* (Langston et al. 1987, 1990). Similar conclusions were drawn by Kure and Depledge (1994) who studied *Mya* in Fyn, Denmark. Here, as in the UK, the extent of TBT uptake reflected proximity to marinas and commercial shipping, and varied seasonally according to boating intensity (BCF 57– 220×10^3). Recent studies have established that this distinctive behaviour persists extensively; body burdens in *M. arenaria* exceeded those in a range of other bivalve and gastropod species sampled in the Bohai Sea, and in wider Chinese seafood markets, usually by at least an order of magnitude (Zhou et al. 2003; Yang et al. 2006).

Bioaccumulation of TBT in *Mya* (occasionally $>50 \mu\text{g g}^{-1}$ [dry wt] near sources) is enhanced by a relatively low rate of metabolism and excretion – with the bulk (80–90%) of total BT in tissues being present as the parent compound (Langston et al. 1987; Kure and Depledge 1994; see also Fig. 16.1). Kinetics of TBT uptake and loss in *Mya* were compared recently alongside those in mussels *Mytilus edulis*, during aqueous exposures (Yang et al. 2006). Accumulation rate constants for TBT in *Mya* (0.54–2.97) were an order of magnitude higher than those in *Mytilus* (0.062–0.3), driven partly by slower losses: during depuration (28 days) the biolo-

gical half-life for TBT in clams was approximately three-fold longer than in mussels. Furthermore the percentage of TBT never fell below 89% of the total BT in clams, consistent with a low rate of degradation.

Viscera and gills of *M. arenaria* were shown to be primary sites for accumulation of TBT during aqueous uptake experiments (Kure and Depledge 1994; Yang et al. 2006), and in the wild (Harino et al. 2005a). *Mya* is an infaunal suspension feeder which ingests small detrital particles and microorganisms which are suspended in the water column just above the sediment, and may also absorb and utilize dissolved organic matter (Stewart 1978). Given the high affinity of TBT for such material, coupled with the high filtration rate of *M. arenaria* (up to 541 day⁻¹), it is easy to see why both digestive gland and gills contribute significantly to the high loadings in these clams. Unlike mussels, however, the composition of BTs in different tissues of *Mya* is variable (Fig. 16.1): the low percentage of degradation products MBT and DBT in the digestive gland of *M. arenaria* suggests that metabolism of TBT may be particularly slow here, contributing to the overall 'efficiency' of accumulation in this tissue.

The relative importance of TBT assimilation routes has not been quantified in *M. arenaria*, although the fact that BCFs measured in experimental (aqueous) exposures are much lower than in the field implies that sediment/diet are important TBT vectors in nature. As a general rule, TBT bioconcentration factors, relative to sediment (BCF_{sed}), are elevated in such deep-burrowing clams, compared with surficial and epibenthic molluscs (Table 16.1). BCF_{sed} in the latter are usually below unity, indicating that body burdens in types such as mussels, winkles and some surface-dwelling bivalves are unlikely to be magnified above those in sediment. In contrast *Mya* and *Scrobicularia* exhibit some of the highest BCF_{sed} in estuarine systems, whilst comparable values have been described in clams *Elliptio complanata* exposed to contaminated sediments from freshwater harbours (Chau et al. 1989; Table 16.1). Offshore, deposit-feeding clams such as the protobranch *Nuculana pernula* have particularly high bioaccumulation potential for TBT (an order of magnitude higher than other filter feeding bivalves and gastropods from the same environment) with BCF_{sed} ranging from ~70 to >400 – highly useful in a monitoring context (Strand et al. 2003). Body burdens in *N. pernula* have been shown to reflect TBT gradients in sediments along shipping lanes – for example in the straits between Denmark and Sweden. This relationship was particularly significant following normalization of sediment TBT concentrations to organic content, implying that it is the TBT adsorbed to the organic fraction which is most bioavailable (Strand et al. 2003).

On the available evidence, both feeding style and habitat are important determinants of bioaccumulation potential in bivalves. Coupled with this, variation in the ability to degrade and eliminate TBT results in significant variation in BCFs (Table 16.1). Notable bioaccumulators of TBT, including *Mya arenaria*, *Nuculana pernula* and the horse clam *Tresus capax*, appear to have reduced capabilities for metabolism of TBT, compared with other bivalves from the same environments (Strand et al. 2003; Horiguchi et al. 2003). Such features contribute to a surprisingly wide variation in TBT accumulation patterns.

16.5 Cephalopods

Data on cephalopod body burdens are not extensive but allow an insight into the scale of organotin bioaccumulation in the deep ocean. For example, Takahashi and co-workers (1997) detected organotin (total BT) contamination up to $\sim 400 \text{ ng g}^{-1}$ wet wt in cephalopods sampled (along with fish, crustaceans, echinoderms and gastropods) in the aphotic bathyal zone (135–980 m) of the continental slope in Suruga Bay, Japan (Fig. 16.2). BT levels were generally lower than those in shallow-water organisms from the same bay (up to $4,000 \text{ ng g}^{-1}$ wet wt in benthic fish), but comparable to those reported in industrialised areas like Tokyo Bay, confirming transport of butyltin pollution to deep-sea ecosystems. Organotin concentrations in cephalopods from Suruga Bay were, in fact, above ‘threshold effects levels’ in the most sensitive molluscs ($\sim 20\text{--}100 \text{ ng g}^{-1}$ in dogwhelks and oysters), and in excess of levels causing cytotoxicity in cultured fish cell lines. However, effects in cephalopods are largely unstudied. In Nuevo Gulf, off Argentina, one sample of the octopus *Enteroctopus megalocyathus* has been recorded with signs of pseudohermaphroditism (Ortiz and Ré 2006) and though the authors indicated that TBT should not be discarded as an explanation for the observed malformation, the absence of consistent observations linking OTs to reproductive impairment in cephalopods suggests they are less sensitive than gastropods and bivalves.

Compared with other deep sea organisms from Suruga Bay, BTs in cephalopods were often comprised of relatively more DBT and MBT than TBT, particularly in the digestive gland (Takahashi et al. 1997), which may indicate an efficient detoxification

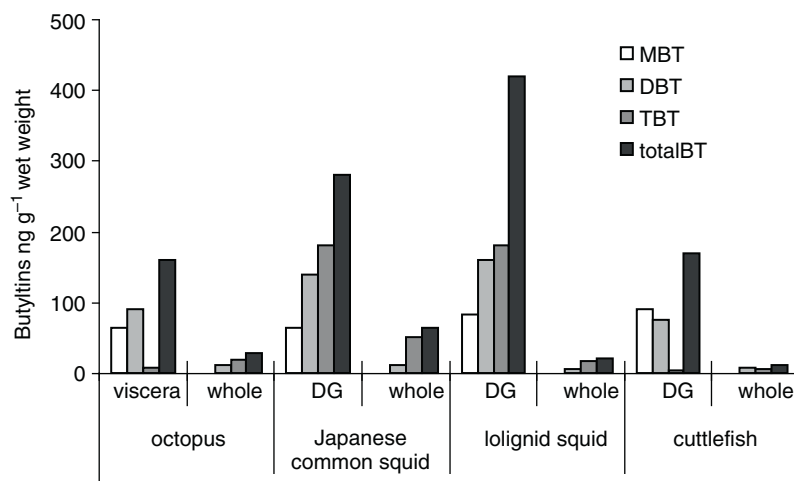


Fig. 16.2 Butyltins in cephalopods from Suruga Bay, Japan (Plotted from data in Takahashi et al. 1997)

system. Nevertheless BTs tend to be concentrated in digestive gland/viscera of squid and octopus, relative to other tissues (Fig. 16.2), implying that the diet is an important uptake pathway.

In more open oceans of the Western Atlantic, deep-sea cephalopods (nine species) have also been reported to contain BTs, but at much lower concentrations (max 2 ng g^{-1} TBT wet wt) than Suruga Bay (Unger et al. 2006). On a larger scale, attempts have been made by Yamada et al. (1997) to use squid as an indicator of OT distributions in the global ocean. The greatest number of samples were from the Sea of Japan and N Pacific, where TBT and TPT concentrations in squid livers increased from ~ 6 and 8 ng g^{-1} , respectively, in the open ocean, to a maximum of ~ 300 and 500 ng g^{-1} , respectively, in coastal waters off Japan (closer to sources and concentrated shipping routes). Estimated BCFs in the digestive gland of the squid *Todarodes pacificus* at seven of these sites ranged between 18,000 and 101,000 (mean 48,000), whilst those of TPT appeared to be even higher ($\sim 500,000$), based on an anticipated, rather than measured values in sea water (Yamada et al. 1997). TPT burdens in squid in this region were generally comparable to, or even higher than, those of TBT, reflecting the history of widespread use of TPT antifouling here. World-wide, TBT concentrations in cephalopods tended to be higher in the northern hemisphere, especially near coasts (up to two orders of magnitude higher), whilst TPT was not detected at all in squid livers collected in the southern hemisphere. These general conclusions must bear the caveats that sampling was spread over 4 years (1989–1993) and that 13 different species were used in different parts of the world. Given the degree of variability seen in other molluscs there is, therefore, some uncertainty over interpretation of trends. Nevertheless the data for squid do present some valuable insights into global patterns in OT distributions.

16.6 Food Chains

Despite the fact that molluscs represent the pinnacle of bioconcentration for OTs, the threat to human consumers appears to be low. As indicated, the ability of molluscs to concentrate OTs is related, partly, to slow rates of metabolism and excretion, whereas most higher organisms possess highly efficient detoxification systems: consequently biomagnification seldom occurs. Only by eating exceptional accumulators of TBT from the most contaminated sites (*Mya* occasionally contains $>20 \mu\text{g TBT g}^{-1}$ dry wt), on a daily basis, would there be a risk of exceeding the tolerable daily intake of $0.25 \mu\text{g TBT kg}^{-1}$ set by WHO. In essence, this gives the 'all clear' for most types of seafood, since tissue burdens are unlikely to surpass those found in *Mya*. Similar conclusions were drawn by Keithly et al. (1999), based on national *per capita* consumption figures and TBT values in marketable seafoods across four continents (which deviated little from an average of $0.185 \mu\text{g TBT g}^{-1}$ dry wt). Where evidence for magnification of OT residues along marine food chains has been presented (e.g. Takahashi et al. 1997) – and

this seems rare – the concentrations involved remain below extreme values seen in *Mya*. It seems it is molluscs themselves, rather than their consumers, that face the biggest risk of harm from tri-substituted OTs.

16.7 Molluscs As Bioindicators of Trends in OT Contamination

The relatively territorial/sedentary habit of most gastropods and bivalves, coupled with other criteria such as longevity, abundance, ease of collection and size, has led to their widespread use in biomonitoring, though usually on regional scales (in contrast to squid whose distribution and mobility may be more valuable in a global context, as described above). Characteristically high BCFs and biological sensitivity to OT, in bivalves and gastropods, has added to their value as indicators. Furthermore, because the kinetics of uptake and loss have been well studied, we have a reasonable insight as to the timescales over which the responses to changing environmental levels can be monitored. The long half-lives of TBT in *Mya*, for example, suggest suitability for measuring longer-term trends, whereas mussels, which have a rapid turnover time, are more amenable to monitoring short-term change.

Monitoring of spatial patterns in OT contamination, based on body burden surveys of molluscs, has been the subject of a large number of regional and national studies – too numerous to describe in detail here. These affirm the significance of expected sources such as marinas, ports and shipping channels (e.g. Shim et al. 1998; Coelho et al. 2002b; Harino et al. 2003; Rato et al. 2006). Of increasing interest in recent years has been the collection of evidence on temporal trends in molluscs – in particular, the contribution of these data towards the arguments for, or against, a global ban on the use of OTs on the commercial fleet, following the earlier restrictions on leisure craft. Often, results from these surveillance programmes indicate that, where partial bans were imposed in the 1980s–1990s, TBT pollution has declined, particularly at open coastal sites. In the UK this is reflected in the recovery of dogwhelk populations in many areas where, previously, pollution was severe (Birchenough et al. 2002). Many oyster fisheries badly affected by TBT have also returned to normal. Nevertheless, TBT pollution has remained a concern in some coastal and estuarine locations where impacted organisms have been slow to recover and levels of TBT in water and sediment can still exceed environmental quality standards and guidelines. Locally, illegal use of TBT paint or disposal of TBT washings may contribute to these apprehensions.

TBT (and TPT) ‘hot-spots’ are usually associated with commercial ports and dockyards, where dredging and disposal of contaminated sediments continues to be problematic for environmental managers because of the threat of remobilisation of OTs. Deposit feeding clams such as *Scrobicularia plana* are ideal candidates for directly assessing the bioavailability of these sediment-bound sources. Even at off-shore sites, particularly those close to TBT-affected merchant shipping routes and anchorages, recovery can be extremely slow due to persistent residues in sediment.

Combined tissue burden data and imposex measurements in gastropod species such as *Buccinum undatum*, *Neptunea antiqua* and *Nassarius (=Hinia) reticulata*, and bioaccumulation assessment in clams such as *Nuculana pernula* are valuable indicators of trends in these offshore locations (Ten Hallers et al. 2003; Strand et al. 2003; Rato et al. 2006).

Partly because of such mollusc-based evidence, regulation of antifouling paints (with specific reference to triorganotins) was recommended by IMO's Environmental Protection Committee in 1999, with a total phase out of organotin antifouling coatings scheduled for January 2008. Ratification of this legislation was announced in 2007, though there are a number of countries which still do not have restrictions on the use of organotin. Even where legislation is in place, there may still be considerable reservoirs of OT in sediments and the potential for remobilisation and impacts on molluscs and other sensitive species may be locally significant during dredging and disposal operations (Chapter 5). Clearly, there may be some way to go before protection of the environment from OTs, and in particular sensitive molluscan components, is complete. Continued long-term monitoring near major ports, dockyards and shipping-routes, using selected mollusc species, would have obvious advantages in determining the consequences of these actions and charting progress towards recovery.

16.8 Conclusions: Lessons Learned from Organotin Bioaccumulation in Molluscs

Despite the negative effects of TBT, there have at least been some useful lessons for ecotoxicologists and regulators, which stem from observations in molluscs. These include the precautionary warning to 'expect the unexpected' in view of the variability in bioaccumulation, persistence and susceptibility between taxonomic groups. Simple models are unlikely to predict risk, universally. The concept that sediments represent an important and long term source of TBT (for some species) has also been established unequivocally, and should provide important indications of the behaviour of other contaminants, under similar scenarios.

Paradoxically, although antifouling paints were developed to combat barnacle settlement, these crustaceans are among the organisms least affected by TBT (Goldberg 1986). The sensitivity of molluscs to the effects of TBT and TPT lies, partly, with their bioaccumulation potential, which itself is a function of two processes – namely uptake and elimination. Uptake of organic contaminants is often considered a reflection of their lipophilicity and transfer across membranes, however there is little evidence that accumulation is purely lipid-dependent in molluscs (Langston et al. 1987; Takahashi et al. 1997) and internal partitioning may be dictated more by an affinity for proteins. Therefore, unlike most lipophilic contaminants, it is not possible to predict taxonomic variability in bioaccumulation of OTs based on simple partition coefficients (e.g. K_{ow}) alone. Bioaccumulation of OT in molluscs is modified by feeding strategy, and will

vary between carnivores, suspension feeders and deposit feeders, according to the bioavailability and concentration in food and sediments, as well as in water. Furthermore, loss rates and depuration times for organotins will vary according to the ability to metabolise and eliminate the parent compound: these rates are likely to depend on a whole gamut of biotic factors, including enzyme activities. Metabolism of TBT is mediated by the cytochrome P450 system, however, the sensitivity of different components of the P450 system to inhibition by OTs appears to be highly variable among molluscs and is likely to result in subtly different modes of action (see review in Langston 1996). The most pertinent example of this involves imposex development in gastropods *Nucella lapillus* which arises as a result of the inhibition, by TBT, of the P450-dependant aromatase responsible for conversion of testosterone to oestrogen (Spooner et al. 1991).

Because of the diversity in uptake pathways and loss processes, BCFs reported for molluscs are extensive in range (Table 16.1). Expressed as biological partition coefficients K_b ($=\log$ BCF), values range over almost three orders of magnitude – from 2.6 – 3.9 in herbivorous gastropods like *Littorina littorea* to 5.6–6 in filter feeders such as *Mya arenaria* and *Dreissena polymorpha*. Given the large diversity of molluscs, our understanding of the causes of variability in organotin uptake and elimination processes is probably still far from complete. This unpredictability highlights a more general issue in ecotoxicology, in that many taxonomic groups are likely to be under-represented (and, possibly, under-protected) by current knowledge levels and research effort.

It can be argued that the effects of TBT on molluscs and other non-target organisms were not predicted because there is a narrow approach to risk assessment, based on a standardized suite of toxicity and bioaccumulation tests, with a limited range of organisms. This is, understandably, due to regulatory and financial constraints. Nevertheless, given the serious nature of TBT contamination, and associated costs in resolving the problem, it seems crucial to broaden the ecological relevance of risk assessment, by expanding the mechanisms and end points examined. Molluscs are an obvious key group to consider for greater inclusion in future assessment programmes. Without this broader approach, perception of risk will become dominated by a rather narrow doctrine, focusing on a few end points and mechanisms which may not be sufficiently protective of the more sensitive ecosystem components.

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