THE BIOAVAILABILITY OF SEDIMENT-BOUND TRIBUTYLTIN (TBT)

By

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Abstract

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Tributyltin is arguably the most toxic compound ever to be deliberately introduced into the marine environment as an ingredient of antifouling paints. It has had widespread toxic effects on a range of marine organisms, with some gastropod species being particularly sensitive. Effects of TBT on non-target species have resulted in partial bans on its use in many countries, so that new inputs to the water column have decreased in most areas.

One of the physicochemical features of TBT is that it is readily sequestered by suspended particulates due to its low solubility and its hydrophobicity, therefore becoming incorporated into estuarine sediments. The availability of this sediment-bound TBT has been investigated through its potential for re-release back to the water column, and directly from the sediment using the sediment dwelling gastropod *Hinia reticulata*.

The sorption process itself has been investigated using natural components to determine the sediment-water partition coefficient (K_d) together with factors affecting its magnitude. Sorption by sediments has been shown to be rapid (minutes), although the achievement of equilibrium may take longer (hours), and exhibits a Freundlich-like dependence on the TBT concentration due to the variable energies of TBT sorption sites on sediment particles. The major determinant of K_d is sediment type, greater adsorption occurring in fine-grained organic rich sediments compared to low organic sands; although both salinity and pH modify the degree of adsorption. The sorption process has been shown to be reversible, so that previously contaminated sediments may act as reservoirs of TBT, releasing the compound back to the overlying water for many years.

Hinia reticulata has been shown to be an effective and quantitative accumulator of both dissolved and sediment-bound TBT, principally acquiring TBT from water across the respiratory surfaces. When additionally exposed to sediments, significantly higher body burdens were accumulated, with up to 80% of the total attributable to the sediment. Uptake of TBT across the surface of the head/foot appears to be an important pathway for sediment-exposed *Hinia reticulata*, while the ingestion of contaminated sediment does not appear to occur. *Hinia reticulata* is capable of metabolising TBT to lesser butylated and presumably less toxic products which are excreted, making its accumulated body burdens responsive to changing environmental TBT levels, and increasing its value as a biomonitor

When exposed to a range of TBT contaminated sediments, *Hinia reticulata* showed there to be greater TBT availability from sediments with a low sorptive capacity (sands), principally through desorption of TBT to the overlying water. Fine-grained organic-rich muds, which have a greater capacity for TBT, produced lower accumulated burdens in *Hinia reticulata*, but may represent more important long-term sources of TBT to benthic organisms in estuaries.

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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1 General Introduction

In recent years the occurrence and effects of tributyltin (TBT) in the marine environment have been widely investigated. TBT use, primarily as the active agent in antifouling paints has led to high concentrations in many marine waters, notably near harbours and marinas. Deleterious effects of TBT, particularly on non-target marine organisms have also been reported worldwide, with effects on the sexuality of neogastropod molluscs being perhaps one of the most sensitive bioindicators of TBT contamination. As a result of these widespread toxic effects, legislation has been enacted in many countries to regulate the use of TBT based paints in an attempt to reduce further inputs of TBT to the marine environment. However, the physicochemical behaviour of TBT leads to its adsorption onto particulates and subsequent incorporation into the sediment phase. Although there are many reported values for TBT concentrations in sediments, the adsorptive process whereby TBT is scavenged by sediment particles has been less frequently investigated, often with conflicting results. The potential for re-release of sediment-bound TBT, and the importance of sediments as a future, secondary source of TBT, long after new inputs have ceased is also uncertain. Similarly, the bioavailability and toxicity of sediment-bound TBT have scarcely been investigated.

This research programme has addressed several of these questions concerning the importance of sediment associated TBT. Partitioning and the environmental factors that affect the sorptive process have been extensively studied, and the potential for re-release of TBT from sediments has been assessed. The bioavailability of TBT from natural sediments has also been investigated using the sediment dwelling neogastropod *Hinia reticulata* as a test organism to determine the relative importance of different uptake routes, and how bioavailability may differ with the nature of the sediment. Information on many of these points has been lacking until now, and is essential for the future management of TBT contaminated sediments.

1.1 Organotin Chemistry and Toxicology

The chemistry of organotin compounds, as an organometallic group is complex, and far outside the scope of this thesis (for reviews see: (Neumann, 1970; Poller, 1970; Zuckerman, 1976)). However, a basic appreciation of organotin chemistry is essential in the context of processes discussed later.

Organotin compounds are characterised by the presence of one or more tin-carbon bonds and have the general formula R_nSnX_{4-n}, where R is an alkyl or aryl group, X is an anionic species e.g. chloride, oxide, hydroxide or other functional group, and n is numerically 1 to 4. Attempts were made for a long time to relate the chemistry of the C-Sn bond to a few simple and constant factors, such as the electronegativity of the tin atom, and thus the polarity of the C-Sn bond (Neumann, 1970). Consequently, the results were of limited validity, and their interpretation was often unsatisfactory. Today, the C-Sn bond is known to be rather long (0.217 nm) and sensitive to the influence of substituents, reaction products, and solvents. The bond is stable below 200°C and resistant to oxidation. However, most importantly the C-Sn bond is easily polarised, and that polarisation may occur in either direction such that the number of possible polar and radical reactions is large (Neumann, 1970).

Industrial manufacture of organotin compounds comprises two principal steps: firstly making direct tin-carbon bonds in compounds such as R_4Sn; while the second involves co-proportionation in which R_4Sn is reacted with tin chloride to produce compounds of the type R_3SnCl, R_2SnCl_2 and $RSnCl_3$. Other derivatives may then be simply produced from these chlorides for industrial end uses.

The number of C-Sn bonds has a profound effect on the properties of organotins allowing a wide range of applications within the group. Tetra-organotins R_4Sn are usually colourless liquids, thermally stable to 200°C and do not react rapidly with air or water. The main uses of these compounds are as industrial precursors for other organotin compounds.

van der Kerk and Luijten, (1954) was one of the first to systematically investigate the properties of organotin compounds, in particular, their fungicidal properties. The toxicological properties of organotins follow a complex pattern, but in general, progressive introduction of organic groups at the tin atom in any series R_nSnX_{4-n} produces a maximum biological activity when n = 3, i.e., for the triorganotin compounds R_3SnX (Blunden et al., 1984). Furthermore, within any R_3SnX series, the toxicity to different biotic species is markedly dependent on the nature of the organic group, as indicated in Table 1.1 (Blunden et al., 1984). In contrast, the nature of the inorganic radical, X, generally has little effect upon the biological activity of the compound (van der Kerk and Luijten, 1954).

Table 1.1 Species specific toxicity of triorganotin compounds.

{From Blunden et al. (1984)}.

Species	R in most active R_3SnX compound
Insects	Methyl-
Mammals	Ethyl-
Gram-negative bacteria	Propyl-
Gram-positive bacteria, fish, fungi, molluscs and plants	Butyl-
Fish, fungi, molluscs	Phenyl-
Fish, mites	cyclo-C_6H_{11}, $PhMe_2CCH_2$

The broad spectrum of acute toxicity shown by triorganotins is thought to be due to disruption of mitochondrial functions by:

- Interaction with mitochondrial membranes causing swelling and disruption;
- Secondary effects derived from their ability as ionophores to disrupt mitochondrial function through mediation of Cl^-/OH^- exchange across the lipid membrane;
- By their ability to inhibit the fundamental processes involved in the synthesis of ATP e.g. mitochondrial oxidative phosphorylation and also photosynthetic phosphorylation in chloroplasts.

Di-organotins are generally considerably less toxic than the triorganotins, and their principal uses are as stabilisers to heat, light and weathering in the plastics industry. Several forms have been shown to be effectively non-toxic and are approved for use in the food industry e.g. clear plastic bottles and plastic film packaging. Dibutyltin compounds are used as stabilisers for non-food contact PVC and as catalysts in the manufacture of polyurethane foams.

Mono-organotins exhibit very low toxicity and their main uses are as synergistic additives in PVC stabilisation, and as esterification catalysts.

1.2 Biofouling and Antifouling

The growth of organisms on the immersed surfaces of marine structures such as ships, boats, buoys, offshore platforms and aquaculture cages can cause a loss of efficiency in the operation of these structures in a process generally referred to as biofouling. The total number of fouling species exceeds 2000 (Evans, 1970) including algae, molluscs (mainly mussels), Crustacea

(barnacles), tubeworms, hydroids and sponges (Evans and Smith, 1975; Simmonds, 1986). The effect of biofouling is an increase in surface roughness which produces turbulent, rather than laminar flow over the immersed structure, resulting in increased frictional drag and acoustic noise (Champ, 1986). In the case of ships and boats, this leads to a degradation in performance, and an increase in fuel consumption (Schatzberg, 1987). As little as a 10-micron increase in hull roughness can result in a 0.3 - 1.0% increase in fuel consumption (Champ and Lowenstein, 1987). Thus, using the QE II as an example (Champ and Lowenstein, 1987), the fuel costs for that ship in 1985-86 amounted to about $17 million, such that a 1% increase in fuel consumption equated to $170,000 for the year. In addition there is the cost of dry-docking, or underwater hull cleaning to remove the fouling organisms, with a consequential loss of operational time. The U.S. Navy, with its 600 ship fleet, estimated the annual cost of biofouling to be in excess of $110 million (Champ and Lowenstein, 1987).

Methods to counteract biofouling have been reported from as early as 300 BC when lead sheets were used to cover the wooden hulls of ships to inhibit boring rather than fouling organisms. From the 17th century onwards, copper sheeting was used as the principal safeguard against the shipworm *Teredo* (Stebbing, 1985). The first antifouling paints were introduced at the beginning of the 20th century (Champ and Pugh, 1987) consisting of a matrix, a pigment and a powerful biocide (Champ, 1986). Small amounts of the biocide were released at the paint surface to kill any settling organisms.

Since the introduction of antifouling paints many compounds have been utilised as biocidal agents. Copper salts were among the first to be used (Christie and Dalley, 1987) while organomercury and stereoarsenicals were also added to increase the effectiveness of copper salts (Stebbing, 1985). However, in the 1970's their use was stopped because of their high toxicity and levels of environmental contamination (Champ and Pugh, 1987).

The most effective biocides produced in recent years have been the organo-metallic compounds of arsenic, lead, mercury and tin (Christie and Dalley, 1987), with the organotins originally thought to be the most acceptable toxicologically and environmentally. Organotins had previously been used as fungicides and preservatives for wood, textiles and paper (Champ and Pugh, 1987; Goldberg, 1986). Tributyltin (TBT) compounds were first introduced into

antifouling paints in the early 1960's although they were not used in significant quantities until 10 years later (Stebbing, 1985).

TBT has been utilised as a biocide in antifouling paints in three different ways: in *free-association* paints; in *ablative* paints; and in *co-polymer* formulations. In free-association paints the TBT is not chemically integrated with the paint matrix and simply leaches out by contact with seawater. This results in a high early release rate coupled with a short period of protection from fouling (approximately 2 years) since calcium carbonate gradually clogs the microchannels in the paint surface, inhibiting the release of biocide. Consequently, some of the biocide may remain unused in the paint, which, when the hull is scraped prior to repainting may re-enter the environment as paint flakes or chips. Ablative paints feature a similar chemistry except that the matrix sheds during use as the paint surface roughens, flaking off in thin microlayers exposing a fresh surface and more biocide underneath. This type of paint also has an effective lifetime of about 2 years (Champ and Lowenstein, 1987). Copolymer paints were developed in the early 1970's and were of a different formulation to the previous types. The TBT is chemically bonded to a polymer backbone, which breaks down in the presence of water under slightly alkaline conditions - just the circumstances found in seawater. As the polymer breaks down, the surface of the paint erodes exposing more copolymer below. Since release of the biocide is governed by chemical decomposition of the TBT group rather than dissolution of the paint particles release rates from copolymer paints are typically lower than for the other types (except for a short period after initial application). The slow hydrolysis of the polymer gives copolymer paints a prolonged, constant and low release rate resulting in a longer and more effective lifetime of between 5 and 7 years (Champ and Lowenstein, 1987). In addition, copolymer paints can be re-applied without having to remove previous copolymer layers so the shipyard costs are less, and there is reduced input to the environment from scraping. The difference in the various types of TBT paints has important considerations in the regulation of TBT discussed later.

Tributyltin based paints have proved to be amongst the most effective antifouling formulations ever developed, due to their toxicity, effective lifetime, and the nature of the constantly renewed surface. Additional benefits include the fact that unlike copper based paints, there is no galvanic corrosion when TBT paints are applied to aluminium hulled vessels (Karpel, 1988);

and the colourless nature of TBT means that paints of almost any colour could be formulated, in contrast to the dull reds, browns and blues of copper based antifoulings.

Thus, for the aquatic environment, organotin-based antifouling paints have represented the most important source of TBT.

1.3 Environmental Occurrence and Fate of TBT

The toxicological impact of TBT, reviewed later, has been the main reason for environmental monitoring programmes, biogeochemical pathway studies, and investigations of the degradation processes for this contaminant. Concern over its presence in the environment in the 1980's led to development of a wide range of analytical techniques for its determination. Most methods were complex multi-step protocols involving extraction, clean up, derivatization, separation and finally detection. Such protocols, although sensitive were not always accompanied by quality control data, such that although there is an extensive array of data available for environmental levels of TBT, the comparison of data between different research groups, laboratories, and countries must consider this analytical uncertainty. Only in recent years have certified reference materials (CRMs) for butyltins become available, and then only for limited environmental and biological matrices (sediments and fish tissue). Nevertheless, environmental levels of butyltins especially TBT are reviewed here, together with data on bioaccumulation, toxicity, and environmental fate. This is not an exhaustive review, since the published data world-wide are too extensive to be summarised here, and the purpose in this context is to describe the environmental levels that have been recorded and the effects which have been seen in organisms exposed to environmental contamination.

1.3.1 Water and the Surface Microlayer

Since its first use in antifouling paints in the 1970's, TBT has been extensively monitored worldwide. Data summarised in Table 1.2 show that recorded concentrations in the water column vary considerably, from less than 1 ng l^{-1} to over 30,000 ng.l^{-1} TBT recorded in water from a ship repair yard in Bahrain (Hasan and Juma, 1992). As a general summary of these results the highest concentrations of TBT were found in marinas and harbours. Waldock et al. (1987) studied 9 estuarine sites in the UK on a monthly basis during 1986 and showed

negligible riverine input of TBT. Most of the sites studied exceeded the UK EQT at that time (20 $ng.l^{-1}$ TBT) while the highest levels were found in marinas or harbours which had restricted water exchange with the open sea e.g. poorly flushed waters of Sutton Harbour in Plymouth contained over 1000 $ng.l^{-1}$ TBT compared with the well-flushed Dart marina where water concentrations were found not to exceed 200 $ng.l^{-1}$. Similar characteristics have been observed by other authors: Valkirs et al. (1986b) monitored waters in San Diego Bay between 1983 and 1985 and recorded a 10 –19 fold increase in TBT levels in the water of a yacht basin harbouring about 2000 yachts.

Table 1.2 Concentrations of tributyltin (TBT) in marine and estuarine waters

Region	Date	Location	Filtered	Concentration (ng l^{-1} TBT)	Analytical Method	Reference
UK Southwest England	1984	Estuarine & coastal waters		<100 – 875*	GFAAS	(Cleary and Stebbing, 1985)
UK Southwest England	1986	Estuarine & coastal waters		<20 – 723*	GFAAS	(Cleary and Stebbing, 1987)
UK Scotland	1986	Sea loch near TBT treated fish farm cages		2500* after 1 day 200* after 15 days 35* after 8 weeks 5* after 5 months		(Balls, 1987)
UK Poole Harbour	1985 - 1987	Poole harbour Marina		5 – 348* 585 – 1590*	GFAAS	(Langston et al., 1987)
U.K. Estuaries	1986	Crouch Blackwater Beaulieu Poole Teign Dart Salcombe Milford Haven Menai Straits Conwy Plymouth Brighton		<1 - 425 <1 - 831 <1 - 146 <1 - 1520 <1 - 57 <1 - 191 <1 - 264 <1 - 290 <1 - 3 ng <1 - 9 ng 83 - 1270 27 - 879	GC-FPD	(Waldock et al., 1987)

All data refers to unfiltered water samples except where indicated.

* Refers to data converted from ng l^{-1} TBT as Sn in original publication (divide by 2.4 to convert to ng l^{-1} TBT as Sn).

Continued...

Table 1.2 (continued) Concentrations of tributyltin (TBT) in marine and estuarine waters

Region	Date	Location	Filtered	Concentration (ng l^{-1} TBT)	Analytical Method	Reference
UK Estuaries	1985 - 1990	South West England		<2.5 – 115*	GFAAS	(Langston and Burt, 1991).
		South Coast		1.5 – 883*		
		East Coast		<2.5 – 129*		
UK	1990 - 1992	Estuarine and near-coastal waters		<1 - 35	GC-FPD	(Law et al., 1994)
		Offshore waters		<0.5 - 12		
UK	1988 - 1989	Estuarine & coastal waters		45 - >250*	GFAAS	(Cleary, 1991)
SW England						
Denmark	1988 - 1989	Marinas		60 – 945*	GFAAS	(Kure and Depledge, 1994)
		Fjords & open water		<25*		
France	1995	Côte d'Azur		<0.6 - 459	GC-FPD	(Tolosa et al., 1996)
Netherlands	1989	Reference Harbours	0.45µm	0.1 - 15.0	GC-AAS	(Ritsema et al., 1991)
		Harbours		0.1 - 76.0		
		Marinas		110.0 - 3620		
France	1986 - 1987	Marinas & oyster culture areas		<2 - 1500	HGAAS	(Alzieu et al., 1989)
Atlantic coast						
France	1988 - 1989	Harbours		2 – 833	HGAAS	(Alzieu et al., 1990)
Mediterranean coast		Marinas		18 - 736		
		Mariculture areas		<2 - 111		
Belgium	1991	Harbour waters		10 - 443	GC-AAS	(Dirkx et al., 1993)
Antwerp harbour					GC-AED	
Rhine river	no data	Harbour waters (non-saline)		25 – 182.5*	HGAAS	(Schebek et al., 1991)
Freshwater						

Continued...

Table 1.2. (continued) Concentrations of tributyltin (TBT) in marine and estuarine waters

Region	Date	Location	Filtered	Concentration (ng l^{-1} TBT)	Analytical Method	Reference
Spanish Mediterranean Coast	1988	Marinas	GFC	74 - 369	GC-FPD	(Tolosa et al., 1992)
		Harbours		14 - 30		
		Sewage disposal site		11 - 16		
		Shellfish culture area		<2 - 17		
German Bight	1990	Coastal and offshore waters		0 - 11.9	GFAAS	(Hardy and Cleary, 1992)
Netherlands	1988 - 1989	Marinas / Harbours		0.1 - 7210	GC-FPD	(Ritsema et al., 1991)
Mediterranean	1988	Spanish coast	GFC	<20 - >360	GC-FPD	(Alzieu et al., 1991)
		French Midi coast	Unfiltered	<20 - >800	GC-FPD	
		French Riviera, Monaco & Italian coast	Unfiltered	<20 - >1700	GC-FPD	
Mediterranean N. Tyrrenian Sea	1988	Harbours		<20 - 810	GC-FPD	(Bacci and Gaggi, 1989)
		Marinas		45 - 3930		
		Power plant		11930 - 12150		
Greece	1987 - 1988	Marinas		62.5 – 1058*	GFAAS	(Fytianos and Samanidou, 1990)
Egypt	no data	Alexandria Harbour	GF/C	16 - 74	GC-FPD	(Abdallah, 1995).
			Unfiltered	18 - 83.5		
Bahrain	no data (1991?)	Marina		9375 – 10750*	GFAAS	(Hasan and Juma, 1992)
		Commercial dry-dock		6975 – 13700*		
		Ship repair yard		13392 – 33375*		
		Yacht club marina		12575 – 15750*		
U.S.A. Chesapeake Bay	1985 - 1986	Marinas		<20 - 998	GC-FPD	(Hall et al., 1986)
		Harbour		<20 - 112		
		Rivers		<20 - 24		
		Shipping channel		<20		

Continued...

Table 1.2. (continued) Concentrations of tributyltin (TBT) in marine and estuarine waters

Region	Date	Location	Filtered	Concentration (ng l^{-1} TBT)	Analytical Method	Reference
San Diego Bay	1983 - 1985	Commercial Basin		10 - 550	HGAAS & GFAAS	(Valkirs et al., 1986b)
U.S.		Shelter Is. Yacht Basin		20 - 930		
		Reference Stations		<10 ng		
USA	1987	Marina areas		34 - 367	GC-FPD	(Matthias et al., 1988)
Chesapeake Bay						
USA		Marina areas		291 - 4435	GC-FPD	(Hall et al., 1987)
Chesapeake Bay						
USA		Spa Creek		22 - 530	GC-FPD	(Batiuk, 1987)
Chesapeake Bay		Back Creek		19 - 170		
		Trend Avon river		23 - 60		
		Plain Dealing Creek		16 - 91		
USA	1986	Marina		5 - 47	GC-FPD	(Huggett et al., 1986)
Southern Chesapeake Bay		Private marina		9 - 98		
		Rural creek		5 - 20		
		York River		<1 - 8		
Chesapeake Bay	1987	Marinas		142 - 367	GC-FPD	(Matthias et al., 1988)
U.S.		Severn River		33 - 35		
New South Wales	1987	Sydney Harbour &		10 – 550*	GC-ECD	(Batley et al., 1989)
Australia		Georges River				
New Zealand	1987	Estuaries & marinas		<40 – 803*	HGAAS	(King et al., 1989)
Hong Kong	1988 - 1989	Marinas		<90 - 1000	GC-FPD	(Lau, 1991)
		Typhoon shelters		234 - 510		
		Mariculture sites		<90 - 285		
Peninsular Malaysia	1992	Open Water	0.45µm	<3.4 - 20	GFAAS	(Tong et al., 1996)
		Estuarine & port areas	0.45µm	30 - 282		

Cleary and Stebbing, (1987) showed pleasure craft rather than commercial shipping to be the predominant source of TBT in SW England, while Alzieu et al. (1990) demonstrated that higher concentrations of TBT were to be found in French Mediterranean marinas compared to marinas on the Atlantic French coast, which was attributed to reduced tidal flushing in Mediterranean sites.

Several authors have also reported seasonal variation of TBT concentrations in water. Fortnightly sampling over a period of 1 year at Burnham-on-Crouch, demonstrated that highest concentrations occurred during the spring, corresponding with the launching of freshly antifouled boats (Waldock, 1986). Furthermore, a decline in concentrations was recorded over the summer, followed by a second peak in late summer, probably as a result of boat cleaning prior to a local regatta in August. Similar temporal changes were also demonstrated in the Elizabeth River, Virginia (USA), with peaks attributed to the painting of cruise ships in December (Seligman et al., 1986b).

Cleary, (1991) carried out a tidal cycle study to determine the effect of tidal change on TBT concentrations. Results over a 48-hour period at Sutton harbour, Plymouth, showed that maximum concentrations occurred at low water (128 $ng.l^{-1}$ TBT) and minimum values at high water (52 $ng.l^{-1}$ TBT) due to the dilution effect of incoming, less contaminated seawater. A similar effect was also found in the river Crouch (Waldock et al., 1987) where a two-fold change in TBT concentrations occurred during a tidal cycle. Tidal exchange near the mouth of a yacht basin in San Diego Bay (USA) resulted in 20-fold changes from 17 – 340 $ng.l^{-1}$ TBT (Clavell et al., 1986). Clearly the influence of tidal changes depends very much on local conditions.

Not all TBT inputs however have been solely attributable to ships and boats. Although negative TBT concentration gradients were measured away from a marina in Western Mediterranean coastal waters, significant increases in an offshore area of sewage disposal were also seen, suggesting that some TBT may be of urban/industrial origin in some instances (Tolosa et al., 1992).

From Table 1.2 it is apparent that very high TBT concentrations occur near dry docks and ship repair facilities, as the direct result of shipcleaning and repainting. Dirkx et al. (1993) measured

TBT concentrations in the waters of Antwerp harbour – a large harbour with very limited water exchange – and found that concentrations generally ranged from 10 to 45 ng.l^{-1} TBT, but increased up to 440 ng.l^{-1} in areas where ships were hosed down prior to repainting.

In contrast, sampling of dry-dock and harbour waters during and after the painting and undocking of the USS 'Badger' in Pearl Harbour naval shipyard, where comprehensive efforts had been made to prevent entry of TBT to the environment by paint overspray etc., showed that of 68 kg TBT applied to the ship, only 15 g were estimated to have been released during undocking, with the result that there was no measurable increase in water concentrations near the shipyard 2 days later, demonstrating that preventative measures, though costly, can be very effective (Adema et al., 1988).

Besides its use on ships, TBT has been employed as an antifoulant in industrial cooling waters. TBT concentrations were measured in harbour and marina waters from the Northern Tyrrenian Sea, Italy, including a harbour receiving TBT contaminated coolant from a thermoelectric power plant utilising approximately 7 kg TBT per day. This resulted in concentrations of approximately 12000 ng l^{-1} TBT at the power plant outlet, although these very high levels were reduced to <20 ng.l^{-1} 1km offshore (Bacci and Gaggi, 1989).

TBT has also found applications in the aquaculture industry, where fouling of cages and netting can be a major problem. The leaching of TBT from freshly treated salmon farm cages that had been coated with a free-association TBT compound has been assessed (Balls, 1987). TBT concentrations were approximately 2500 ng.l^{-1} TBT within the cage soon after deployment, but dropped to 200 ng.l^{-1} TBT after 2 weeks, and 5 ng.l^{-1} TBT after 5 months.

The surface microlayer of natural waters, which contains a hydrophobic film of long-chain fatty acids, alcohols, esters and other compounds is thought to be important in the aquatic environmental distribution of hydrophobic pollutants. The thickness of the microlayer, which is believed to exist as a monolayer, is in practical terms defined by the type of sampling equipment used (Garrett, 1965). Considerable evidence has accumulated to demonstrate that TBT is enriched in the surface microlayer of marine and estuarine waters compared with the underlying bulk water. This is not surprising given that many organic compounds accumulate as surface films and may act in a manner analogous to the solvents used in many analytical

Table 1.3 TBT concentrations and enrichment in the surface microlayer compared to sub-surface water.

Region	Location	Year	Sampling Method	Surface Microlayer Concentration (ng l^{-1} TBT)	Sub surface Concentration (ng l^{-1} TBT)	Enrichment Factor	Analytical Method	Reference
Chesapeake Bay U.S.	Small Marinas Large Marinas	1985 - 1986	Teflon Sheet	29 - 1049 34 - 1049	51 - 123 119 - 408	2.2 - 25	GC-FPD	(Hall et al., 1987)
Chesapeake Bay U.S.	Marinas Severn River	1987	Glass Plate	143 - 5980 50 - 70	142 - 367 33 - 35	0.8 - 11.2	GC-FPD	(Matthias et al., 1988)
Canada & New York State USA	74 locations (Freshwater)	no data	Glass Plate	456 – 1135200*	24 – 4032*	41 - 47300	GC-AAS	(Maguire and Tkacz, 1987)
SW England	23 Harbour & Estuarine locations	1986	S/S Wire Screen	57.6 – 2566*	<19.2 – 694*	1.9 - 26.9	GFAAS	(Cleary and Stebbing, 1987)

* Refers to data converted from ng l^{-1} TBT as Sn in original publication (divide by 2.4 to convert to ng l^{-1} TBT as Sn).

procedures to extract TBT from seawater. These natural organic sea surface films are monomolecular layers, polar in nature, which act as sites for the accumulation of lipophilic pollutants (Garrett and Duce, 1980). The lipophilic moiety of the TBT molecule which is responsible for its toxicity, and confers its ability to partially dissolve in fatty tissue, also results in its hydrophobic nature, which inherently favours its accumulation at the air-water interface. The hydrophobic nature of TBT has been reported (Laughlin et al., 1986b) and the octanol-water partition coefficient (log K_{ow}) of TBTO found to be between 3.8 in de-ionised water and 3.7 in water of 25‰ salinity. Typically log K_{ow} values in excess of 3 classify a compound as lipophilic, i.e. likely to accumulate in the fatty tissues of aquatic organisms (Connell, 1988).

It has been suggested that microbubbles in the water column, generated by surface wind stress, may act as a scavenging mechanism for TBT in the water column, and that upon their rise to the surface, bubbles lead to surface microlayer enrichment of TBT, although bubble bursting with attendant jet and film drop ejection may also act as a depletion mechanism for TBT (Gucinski, 1986).

Given that the surface microlayer is thus enriched with TBT the community of organisms that inhabit this layer for all, or part of their lives would be most at risk. This assemblage of species includes micro-organisms, some adult and larval invertebrates, together with some fish eggs and larvae. Furthermore, littoral organisms will be periodically bathed in the microlayer upon rise and fall of the tides, as will the substrata upon which they live and graze. Since TBT readily adsorbs onto surfaces, organisms that move over them may be exposed to higher levels than water concentrations alone may suggest.

Table 1.3 summarises some of the data collected for microlayer enrichment of TBT.

The range of microlayer concentrations, and enrichment factors reported are variable, as the result of several factors:

- The variability of hydrophobic components in the surface layer, e.g. alcohols, fatty acids, hydrocarbons, proteins and carbohydrates;
- The different sampling techniques employed. These have included stainless steel wire screens (Cleary and Stebbing, 1987); glass plates (Maguire and Tkacz, 1987); and teflon

sheets (Hall et al., 1987), each of which removes a layer of different thickness, such that direct comparison between methods is not possible.

Furthermore, it is interesting to note that the enrichment factors for estuarine and marine waters are considerably lower than those for freshwater (Maguire and Tkacz, 1987), which may reflect both a different solubility of TBT in freshwater, and the nature of the surface film. However, all results suggest that surface microlayer enrichment of TBT occur to varying degrees in natural waters.

1.3.2 Sediment-Water Partitioning

The equilibrium partitioning of contaminants between sediments and water is described by a partition coefficient, K_d, whose value is generally determined by laboratory experiments with natural sediments and water. An apparent partitioning coefficient may be calculated from concentrations measured in field sediments and water, but this will only approach the equilibrium K_d value in cases where the sediments are in equilibrium with the overlying waters. On the basis of its octanol water partition coefficient (5500 – 7000 (Laughlin et al., 1986b)) TBT would be expected to partition to organic matter in suspended solids and sediment. A further complication is that the binding of a polar molecule such as TBT may be a function of its hydrophobicity, due to the butyl groups, and its polarity, so that factors other than organic content alone may be important. Measurements of apparent K_d values in field samples support this, and show considerable variation due to a range of factors including organic content, grainsize (though surface area is likely to be more relevant), salinity and pH. Laboratory experiments (Harris and Cleary, 1987; Macintyre and Smith, 1984; Randall and Weber, 1986; Stang and Seligman, 1987; Unger et al., 1988) have investigated effects of some of these parameters, but Batley, (1996) considers that there is insufficient information to enable a full interpretation. Hence the importance of this study to understand the partitioning of TBT, and determine the influence of environmental factors on its behaviour in estuarine systems.

1.3.3 Sediment

Partitioning and removal of TBT to particulates, as indicated by sediment-water partition coefficients, result in sediment TBT concentrations several orders of magnitude higher than in

the water column. Table 1.4 summarises some of the TBT concentrations, which have been reported worldwide. Once again this is not an exhaustive list, but serves to illustrate salient features. Although sediment concentrations vary widely, even within individual estuaries, lowest concentrations have been reported from offshore areas e.g. (Stewart and Thompson, 1994) and increased levels have been reported from most estuarine and coastal areas (Dowson et al., 1992; Langston and Burt, 1991; Wade et al., 1988) where vessels, antifouled with TBT based paints were found. Furthermore, there has been a general pattern of high concentrations in sediments from marina areas, since these contain high densities of pleasure craft, and in many instances have poor water exchange with the open sea so that high concentrations of leached TBT can accumulate and be sequestered by the sediment phase. For example, Langston et al. (1987) found TBT levels of around 50 ng g^{-1} TBT near the mouth of Poole Harbour, UK, while at a marina in the inner harbour sediment TBT levels reached over 1200 ng g^{-1} TBT due to poor tidal flushing. Areas subject to large vessel traffic may however be less impacted than areas where small vessels predominate, since large ships spend a greater proportion of their time at sea than in harbour. However, long-term studies of TBT in the Hamble and Itchen estuaries (yacht mooring areas) of Southampton Water, UK have shown decreasing TBT concentrations in water following the 1987 ban, sediment TBT concentrations have declined much more slowly (Langston, 1994). In contrast, there has been no significant decline in sediments or water in the Test estuary (shipping, warships and ship repair facilities) over the same period (Langston, 1994). Krone et al. (1989a) reported sediment TBT levels of 13 – 25 ng g^{-1} TBT in a shipping channel in Puget Sound. The highest sediment TBT levels however, have been reported almost exclusively in, or near to dry docks or other locations where ships are cleaned and repainted e.g. (Demora et al., 1995; Hasan and Juma, 1992; Ko et al., 1995; Tolosa et al., 1992; Yonezawa et al., 1993). Although sediment TBT contamination may generally be high in such areas, there may also be instances where paint flakes, chippings or contaminated blasting grit occur in sediments, resulting in very high concentrations which may not be representative of the area as a whole. For instance, concentrations of almost 130,000 ng g^{-1} TBT were reported in sediments below vessel hoists in Causeway Bay, Hong Kong, but with a significant degree of variation between replicate samples attributed to high-concentration clusters due to the dispersion of paint flakes (Ko et al., 1995).

Sediments may therefore be significant reservoirs of TBT derived from particulate scavenging (adsorption) in the water column, or by direct incorporation of paint chips. The greater persistence of TBT in sediments compared with the overlying water means that this reservoir may remain as a secondary source of TBT long after new inputs to the water column have decreased as the result of paint bans.

Hence, the information presented in this thesis is of particular relevance in determining the importance of sediment-bound TBT as a secondary source to overlying water, together with its significance as a direct source to sediment dwelling biota.

Table 1.4 TBT concentrations in marine and estuarine sediments.

Region	Date	Location	Concentration (ng g^{-1} TBT)	Analytical Method	Reference
UK Poole Harbour	1985 - 1987	Near harbour mouth	48*	GFAAS	(Langston et al., 1987)
		Marina	1248*		
UK Estuaries	1985 - 1990	South West England	4.8 – 40.8*	GFAAS	(Langston and Burt, 1991)
		South Coast	16.8 – 1512*		
		East Coast	4.8 – 2760*		
UK	1990	East Coast Estuaries	7.2 – 9444*-	HGAAS	(Dowson et al., 1992)
Denmark	1988 - 1989	Marinas	108 – 178*	GFAAS	(Kure and Depledge, 1994)
		Fjords & open water	<24 – 127*		
Portugal	1986	Shipyard	1248*	HGAAS	(Quevauviller et al., 1988)
Sado Estuary		Industrial area	45.6 – 50.4*		
Portugal	1986 - 1988	Ria of Aviero	45.6 – 787*	GC-AAS	(Cortez et al., 1993)
Coastal environments		Tejo Estuary	535*		
		Sado Estuary	24 – 1248*		
		Ria Formosa	2.4 – 40.8*		
Western Mediterranean	1988	Dry-dock harbour	9260	GC-FPD	(Tolosa et al., 1992)
		Marina wharf	2420		
		Sewage disposal site	30 - 120		
Egypt	1988	Alexandria coastal area	72 – 3300*	HGAAS	(Gabrielides et al., 1990)
Canada	1992 - 1993	Benthic sediment	4.6 – 5.3*	GC-MS	(Stewart and Thompson, 1994)
SW British Columbia		(25 km offshore, 377m deep).			
		Intertidal sediment	2.6*		
		Stream sediment	<0.24*		

* Refers to data converted from ng g^{-1} TBT as Sn in original publication (divide by 2.4 to convert to ng g^{-1} TBT as Sn).

Cont....

Table 1.4 (continued) TBT concentrations in marine and estuarine sediments

Region	Date	Location	Concentration (ng g^{-1} TBT)	Analytical Method	Reference
Canada	1981	Port Dover harbour	99 - 159	GC-FPD	(Maguire, 1984)
Ontario		Grand River (mouth)	247 - 371		
		Toronto Harbour	473 - 605		
		Whitby Harbour	90 - 130		
		Kingston Harbour	383 - 435		
U.S.A.	1990	South Portland	24 - 520	GC-FID	(Page et al., 1996)
Maine		Boothbay Harbour	636 - 3900		
Gulf of Mexico	1986 - 1989	not specified	<12 – 214*	GC-FPD	(Wade and Garcia Romero, 1989)
Puget Sound	pre-1989	Industrial & ship repair area	576 – 1632*	GC-MS	(Krone et al., 1989b)
		Ferry terminal and outfall	36 – 156*		
		Shipping channel	12.7 – 24.7*		
		Reference areas	<1.25*		
Southern Chesapeake	1986	Hampton marina	up to 4000	GC-FPD	(Espourteille et al., 1993)
Bay		Elizabeth River	24 - 590		
U.S.	1986 - 1988	East coast	12 – 281*	GC-FPD	(Wade et al., 1988)
Coastal sites		Gulf of Mexico	<12 – 214*		
		West coast	14.4 – 677*		
		Hawaii	158*		
Bahrain	no data	Marina	1073 – 1181*	GFAAS	(Hasan and Juma, 1992)
	(1991?)	Commercial dry-dock	526 – 1325*		
		Ship repair yard	413 – 1368*		
		Yacht club marina	1548 – 2590*		

* Refers to data converted from ng g^{-1} TBT as Sn in original publication (divide by 2.4 to convert to ng g^{-1} TBT as Sn).

Cont....

Table 1.4. (continued) TBT concentrations in marine and estuarine sediments

Region	Date	Location	Concentration (ng g^{-1} TBT)	Analytical Method	Reference
New Zealand	1987	Estuaries & marinas	2.4 – 576*	HGAAS	(King et al., 1989).
New Zealand		Marinas	55 – 802*	HGAAS	(Dernora et al., 1995)
		Commercial wharf	84 – 163*		
		Naval dockyard	9.6 – 3024*		
		Washdown yards	1464 – 3264*		
Hong Kong	1988 - 1989	Marinas	60 - 1160	GC-FPD	(Lau, 1991)
		Typhoon shelters	81 - 1690		
		Mariculture sites	52 - 1100		
		Harbours & coastal	<9 - 400		
		Kowloon Bay	<9 - 189		
Hong Kong	1994	Shipyards & marinas	up to 8400*	HG-QFAAS	(Ko et al., 1995)
		beneath vessel hoists	up to 127200*		
Peninsular Malaysia	1991 - 1992	Beaches & mudflats	<0.7 - 29.3 (wet wt.)	GFAAS	(Tong et al., 1996)
		Port area	216.5 (wet wt.)		
Ise Bay	1987	Ise Bay	2 - 140	GC-FPD	(Yonezawa et al., 1993)
Japan		Shipyard area	540 - 1100		

* Refers to data converted from ng g^{-1} TBT as Sn in original publication (divide by 2.4 to convert to ng g^{-1} TBT as Sn).

1.3.4 Degradation and fate of TBT in the marine environment

The occurrence of TBT in water, the surface microlayer, and sediments reviewed above has considered only the tributyl tin species, although in many instances the concentrations of other butylated tin species have also been reported.

The degradation of organotin compounds may be defined as the progressive removal of the organic groups attached to the tin atom (Blunden and Chapman, 1982):

$$R_4Sn \rightarrow R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4$$

This sequential loss of the organic groups is accompanied by a progressive reduction in biological activity, and was perceived by the formulators of TBT-based paints to be the breakdown route for TBT once released into the marine environment. Determinations of TBT degradation rates are a key element in understanding environmental pathways, risk assessment, and in the development of water quality criteria; and as such form an important part of this thesis.

This degradation through cleavage of the Sn-C bond can occur by a range of processes: UV irradiation, biological cleavage, chemical cleavage, thermal cleavage and gamma irradiation. Of these processes, the latter two are of no environmental significance since the Sn-C bond is stable to about 200°C, and sufficient γ-radiation is unlikely to occur naturally.

The UV spectrum ranges from 10 – 400 nm, just below visible light (400 – 700 nm) although due to air absorption most light reaching the sea surface from the sun is above 290 nm and possesses energy of 300 – 600 kJ mol^{-1}. Since the average Sn-C bond energy is about 210 kJ mol^{-1}, natural solar energy is sufficient to cause bond cleavage since the absorption wavelength of organotins is within the UV region.

Biological and chemical cleavage routes in marine and estuarine environments are largely inseparable, and in each case the transformation pathway from tributyltin (TBT) to dibutyltin (DBT), monobutyltin (MBT) and inorganic tin is the same. Investigation of the photolysis of TBT in freshwater (Maguire et al., 1983) revealed a half-life of 89 days for degradation. However, examination of TBT photolysis and biodegradation in seawater (Watanabe et al., 1992) showed photolysis to be more rapid with a half-life of less than 1 day when there was direct exposure of

the water surface to sunlight and air, although the ambient conditions were far removed from environmental conditions. Under more realistic conditions it was found that degradation of TBT and DBT in unfiltered seawater is relatively fast in summer (half-life of about 1 week), but filtration of the seawater prolonged the half-life to about 80 days implying biodegradation by micro-organisms to be a major process (Watanabe et al., 1992).

Microbial species have been shown to biodegrade TBT. Bacterial and fungal degradation of TBTO has been investigated (Barug, 1981) and the formation of DBT and MBT was found in the presence of carbon sources, but no degradation of MBT was observed. The biodegradation of TBT by natural populations of Chesapeake Bay micro-organisms has been studied (Olson and Brinckman, 1986) and found no significant degradation in the winter, but decreases in TBT concentrations together with increases in DBT and MBT were measured in summer. Furthermore, greater degradation rates were found under incandescent light than in the dark suggesting that photosynthetic organisms may be involved in TBT degradation (Olson and Brinckman, 1986; Seligman et al., 1986a; Seligman et al., 1986b). Microcosm experiments with natural waters showed a dependence of TBT degradation rates on temperature and TBT concentration (Lee et al., 1987; Lee et al., 1989). Degradation half-lives were 6 – 9 days under light conditions, but longer in the dark, supporting the evidence for the role of microalgae such as diatoms and dinoflagellates. Reduced degradation was observed at very high TBT concentrations due to the toxic effect of TBT on some of these organisms. Degradation of radiolabelled TBT has been studied in an enclosed marine ecosystem (a MERL mesocosm) with near natural water column and benthos (Adelman et al., 1990). TBT and its degradation products were monitored for 278 days during which most of the TBT was lost by biodegradation, with approximately two thirds being debutylated to DBT, which was in turn degraded to MBT, while the remaining third was directly degraded to MBT. There was no evidence for the degradation of MBT in the water column.

The fate of TBT, once sequestered by particulates and incorporated into sediments, is more complex. TBT may be degraded through the same pathway as in solution, to DBT and MBT, together with the additional possibilities of re-suspension, desorption or burial into deeper,

possibly anaerobic sediment layers. A further complication is that degradation may occur concurrently with these physical processes (Stang et al., 1992).

Maguire and Tkacz, (1985) showed TBT to be readily taken up by sediments and that there was little release of TBT or breakdown products after 10 months if the sediments were undisturbed; however, the release rate through desorption was a function of the degree of agitation. Sediments where biological degradation was suppressed by the addition of KCN showed no degradation of TBT over 11 months at 20°C.

Stewart and Demora, (1990) concluded that TBT degradation rates in sediments were at least an order of magnitude longer than in the water column, and that TBT degradation processes in sediments were more likely to control the overall persistence of TBT in the marine environment.

Since this is central to the question of the long-term fate of TBT in sediments, degradation rates and loss of TBT from sediments have been investigated as part of laboratory studies discussed in this thesis.

1.4 TBT Legislation and Recovery

The French were the first to introduce restrictive legislation on the use of triorganotins in antifouling paints, banning their use on boats <25 m in length in January 1982 (Alzieu, 1986; Alzieu, 1991). This prompt action followed the evidence that the collapse of the oyster fishery in Arcachon Bay was due to the use of TBT paints on boats in the harbours nearby.

The UK Government was slower to react and the United Kingdom Control of Pollution (Antifouling Paints) Regulations 1985 came into operation in January 1986, restricting the concentrations of tin in dried co-polymer and free-association paints to 7.5% and 2.5% by weight respectively (Abel et al., 1986; Side, 1986). It was not until 1 July 1987 that legislation came into force announcing a ban on the sale and supply of TBT-based antifouling paints and its application to pleasure craft <25 m in length, and on nets and cages used in fish farming (Abel et al., 1987; Duff, 1987) although this was too late to be effective for the 1987 season, thus allowing the use of TBT paints for an additional year.

Similar bans have now been introduced in many countries worldwide.

However, despite these restrictions there are still new inputs of TBT into the environment due to a number of factors. In the UK ships over 25 m in length are still permitted to use TBT based antifouling paints (Abel et al., 1987; Duff, 1987) as are aluminium hulled boats which have been exempted regardless of their size because the alternative, copper based paints cause galvanic corrosion (Karpel, 1988). Illegal use of TBT antifoulings appears to be widespread despite efforts to reduce them (Ambrose, 1994) with boat owners continuing to use them due to their effectiveness and the lack of a comparable alternative.

Evidence from France shows that the introduction of legislation has been effective in reducing environmental levels of TBT, and as a consequence, reducing the levels of effects seen in sensitive organisms. In Arcachon Bay TBT concentrations in seawater decreased by 50% in the first year after restrictions were applied. By November 1986, three years after restrictions, concentrations had been reduced 5 - 10 times (Alzieu, 1986). Recovery was also seen in oysters *Crassostrea gigas* with a decrease in the incidence of shell malformations and a return to normal spatfall (Alzieu, 1986; Alzieu, 1991). Evidence from the UK also suggests that there have been reductions in the levels of TBT in water at most sites following restrictions (Bryan et al., 1993b; Dowson et al., 1993; Langston, 1994; Waite et al., 1991). In addition there is also some evidence of recovery in dogwhelk populations, in Northumbria (Evans et al., 1991) and on the Isle of Cumbrae (Evans et al., 1994).

With restrictions on TBT paints there has generally been a return to copper based formulations, and, in Arcachon Bay for example this has led to an increase in the copper burdens of oysters found close to harbours (Claisse and Alzieu, 1993). In comparison to TBT, copper is much less toxic to oysters (His and Robert, 1987) and other organisms. In addition, copper based paints are not as long lasting as the self-polishing TBT co-polymer paints and there has therefore been renewed enthusiasm for the development of alternative antifouling systems.

1.5 Toxicity of TBT

The lipophilic nature of the TBT molecule leads to an increased capacity for it to be taken up in the tissues of living organisms, compared with inorganic tin. Following the harmful effects observed in oyster culturing areas of France and the UK (Alzieu et al., 1980; Waldock and Thain, 1983) a number of marine ecotoxicology studies were initiated to investigate the

accumulation and effects of TBT on other marine species. Several reviews of butyltin toxicity in aquatic environments have been compiled in recent years (Hall and Pinkney, 1985; Rexrode, 1987). The current literature on TBT toxicity is far too extensive to be reviewed here, but a résumé is included as a brief introduction.

1.5.1 Bacteria and micro-organisms

Dooley and Denis, (1987) investigated the effects of several organotins on the bioluminescent bacterium *Photobacterium phosphoreum* (used in the *Microtox*® bioassay system) and found tributyltin compounds to be more toxic than tetraalkyltins, based on concentrations leading to a 50% reduction in bioluminescence after 5 minutes exposure (EC_{50}, 5 min). These authors also found triphenyltin toxicity to be comparable to TBT toxicity. However, some marine bacteria have been found to be TBT-resistant, including one strain capable of developing in culture media containing 36 mg ml^{-1} TBT (Suzuki et al., 1992) although it has also been reported that TBT concentrations of 5 – 100 µg l^{-1} TBT inhibited colony formation in estuarine bacteria (Uchida, 1993). Tests on organotin toxicity to a marine yeast from Chesapeake Bay demonstrated that in highly polluted water (>250 µg l^{-1} TBT) TBT can lead to decreased cell viability due to increased K^+ losses (Laurence et al., 1989).

1.5.2 Plankton

Algistatic TBT concentrations of 10 µg l^{-1} TBT have been reported for the diatom *Skeletonema costatum*, together with reduced cell division rate in *Dunaliella tertiolecta* and *Pavlova lutheri* at concentrations of 10 ng l^{-1} TBT (Beaumont and Newman, 1986). However, algistatic conditions in *Tetraselmis suesica* occurred at much higher TBT concentrations – 1000 µg l^{-1} TBT (Thain, 1983). 72h EC_{50} values for TBTO in *Dunaliella tertiolecta* have been determined at 0.75 µg l^{-1} TBT (Liying et al., 1990). Growth EC_{50} concentrations of 1 µg l^{-1} TBT and 2.9 µg l^{-1} TBT have been measured for the diatoms *Skeletonema costatum* and *Thalassiosira pseudonana* respectively (Walsh et al., 1985). It was concluded that organotins could pose a threat to survival of phytoplankton in some contaminated ecosystems.

Acute toxicity (LC_{50}) values in the range 0.05 – 2.0 µg l^{-1} TBT have been reported for the planktonic copepods *Acartia tonsa* (Uren, 1983), *Nitocra spinipes* (Linden et al., 1979) and

Eurytemora affinis (Bushong et al., 1988). The mysid shrimp *Acanthomysis sculpta* appears more sensitive to TBTO with chronic effects on reproduction measured at concentrations of 0.024 µg TBT l^{-1} (Davidson et al., 1986). TBT toxicity to the copepod *Acartia tonsa* has been assessed at 0.01, 0.05 and 1.0 µg l^{-1} TBT and showed that egg production rate was reduced at all concentrations relative to controls (Johansen and Mohlenberg, 1987).

Therefore deleterious effects on primary and secondary planktonic production may exist at concentrations comparable to those which have been reported in more TBT-contaminated estuarine and coastal regions (Table 1.2).

1.5.3 Polychaetes

A toxicity test using lugworm *Arenicola cristata* larvae exposed to bis (tributyltin) oxide (TBTO) and tributyltin acetate (TBTA) produced 96-hour LC_{50} concentrations of 4.0 and 8.5 µg l^{-1} (as TBT) respectively, and concentrations of 4.3 µg l^{-1} TBTA (as TBT) caused abnormal morphology (Walsh et al., 1986a). Several developmental stages from embryo to swimming larva were exposed, with the early trocophore and early settled stage being the most sensitive. Juvenile *Neanthes arenaceodentata* were exposed to 0, 10, 50, 100 & 500 ng l^{-1} TBT for 10 weeks to determine survival, growth and reproduction (Moore et al., 1991). Significant mortality (79%) was only noted at 500 ng l^{-1} TBT but growth and reproduction were significantly reduced at 100 and 500 ng l^{-1} TBT. No adverse effects were seen at 50 or 10 ng l^{-1} TBT.

1.5.4 Molluscs

There have been many reports of TBT effects on estuarine and marine molluscs including short-term acute effects, and long-term chronic effects on growth, sexuality, reproduction and calcification.

Following mortalities and shell deformation of oysters in Arcachon Bay, France, (Alzieu et al., 1980) exposed adult oysters *Crassostrea gigas* to water contaminated with TBT-based paint leachates at an estimated concentration of 200 ng l^{-1} TBT and found 30% mortality after 110 days. Similarly, *Crassostrea virginica* exposed to 2.5 µg l^{-1} TBT from paint leachates exhibited 50% mortality after 30 days and 80% mortality after 57 days of exposure (Henderson, 1985). In addition, the oyster condition index was affected at concentrations greater than 40 ng l^{-1} TBT

for 57 days suggesting that long-term survival would be affected at concentrations above this level (Henderson, 1985).

There have been several studies of the effects of TBT on juvenile bivalve growth (Salazar and Salazar, 1987; Stromgren and Bongard, 1987; Thain and Waldock, 1986; Valkirs et al., 1987a) which show that concentrations of approximately 125 ng l^{-1} TBT or less affect the growth of the species studied (*C. gigas, O. edulis, C. virginica, M. edulis, V. decussata*). *Mytilus edulis* appeared more sensitive than the other species while *Crassostrea virginica* and *Ostrea edulis* were more resistant than *Crassostrea gigas*. However, it was considered that these effects of TBT on growth might be overestimated due to the stress induced by experimental conditions (Salazar and Salazar, 1987). However, a trend for reduced growth in mussels *Mytilus edulis* and *M. californianus* has been measured along a gradient of TBT contamination in San Diego Bay, California, ranging from 10 – 925 ng l^{-1} TBT (Stephenson et al., 1986). In addition high mortality rates were observed in juvenile *M. edulis* at all sites along this gradient.

The effects of dissolved TBT on reproduction and the early life stages of *Scrobicularia plana* have been investigated (Ruiz et al., 1994b; Ruiz et al., 1995a; Ruiz et al., 1995b; Ruiz et al., 1995c). Embryos showed an EC_{50} (48 h) of 445 – 495 ng l^{-1} TBT while 2 – 3 mm spat showed an LC_{50} of <3.25 µg l^{-1} TBT. Furthermore, burying activity and juvenile growth were significantly reduced at all TBT concentrations used (nominally 1.25 – 10 µg l^{-1} TBT), and it was suggested that TBT may have caused the disappearance of some *Scrobicularia plana* populations in the UK and a reduction in abundance elsewhere in Europe.

The effect of TBT on gastropod sexuality is one of the major features of organotin toxicology, leading to the development of male sexual characteristics in females, and is reviewed separately in the next section. Considering bivalves: (Thain and Waldock, 1986) observed a predominance of male *Ostrea edulis* in populations exposed to 25 ng l^{-1} TBT for 75 days compared with controls. TBT effects on bivalve sexuality appear less significant than in gastropods although *Ostrea edulis* reproduction may be affected by delayed sexual maturity (Thain and Waldock, 1986).

Shell calcification anomalies in oysters have been a more noticeable, and more widely reported effect of TBT in bivalves (Alzieu et al., 1986; Alzieu et al., 1980). Arcachon Bay on the French

Atlantic coast is a major oyster culture area, producing on average 10 – 15000 tonnes/year of *Crassostrea gigas* and also harbours up to 15000 pleasure boats in summer. As early as 1974 shell calcification anomalies, stunted growth and spatfall failures were observed, the shell deformations severely affecting the commercial value of oysters produced. These shell anomalies were created by a chambering process whereby hypersecretion of a gelatinous substance between shell laminae resulted in shells with a ball-like appearance (Heral et al., 1981). Additionally, observations showed that malformations and stunted growth were more apparent in oysters occurring close to marina areas (Alzieu et al., 1981; Alzieu et al., 1980). The role of TBT in these anomalies was established (Alzieu et al., 1981) and confirmed (Thain and Waldock, 1983) although the exact mechanism of TBT action on calcification remains uncertain but may be affected by concentrations as low as 2.0 ng l^{-1} TBT (Chagot et al., 1990).

1.5.5 Crustacea

48 h and 96-h LC_{50} values of 73.0 and 41.0 µg l^{-1} TBT respectively have been reported for the shrimp *Crangon crangon* (Thain, 1983) and a 96 h LC_{50} of 20 µg l^{-1} TBT has been evaluated for *Palaemonetes pugio* (Walsh, 1986). Other acute effects have included growth limitation of *Gammarus oceanicus* by 30 µg l^{-1} TBT after 8 weeks of exposure, while at 300 µg l^{-1} TBT larval survival was zero (Laughlin et al., 1984).

Sublethal effects in crustaceans have been investigated and include the effect of TBTO and its putative environmental product TBTS on zoea larvae of the mud crab *Rhithropanopeus harrisii* (Laughlin et al., 1983). Growth was significantly reduced at concentrations above 0.9 µg l^{-1} TBT and metamorphosis was reduced, even at the lowest concentrations (0.18 – 0.20 µg l^{-1}TBT).

Leg regeneration in the crab *Uca pugilator* (Weis et al., 1987; Weis and Kim, 1988) exposed to TBT concentrations between 0.5 – 25 µg l^{-1} TBT, showed delays in limb regeneration, and ecdysis following regeneration, together with deformities in the regenerated limbs. Additionally, a reduction in burrowing activity of *Uca pugilator* exposed to 12.5 – 125 µg l^{-1} TBT in the laboratory was also found (Weis and Perlmutter, 1987), which could have important ecological effects if it occurred in the natural environment.

1.5.6 Echinoderms

Walsh et al. (1986b) described the effects of dissolved bis (tributyltin) oxide (TBTO) and bis (triphenyltin) oxide (TPTO) on regeneration of arms by the brittlestar *Ophioderma brevispina*. Arm regeneration was inhibited by concentrations of either compound >0.1 μg l^{-1}, and it was suggested that inhibition was caused by neurotoxicological action of these organotin compounds since regeneration is mediated by the radial nerves of brittle stars. Subtle histopathological effects have also been found in starfish *Lepasterias polaris* which had been fed TBT–contaminated mussels for a 53 day period (Mercier et al., 1994). Contaminated individuals showed smaller mature oocytes and thinner gonad epithelium than control organisms, and there was considerable evidence for the metabolism of TBT to DBT and MBT.

1.5.7 Fish

LC_{50} values of 16 μg l^{-1} TBT (LC_{50}, 96 h) for adult Pogge, *Agonus cataphractus* and 5.5 μg l^{-1} TBT (LC_{50}, 48 h) for *Solea solea* have been reported (Thain, 1983). Bushong et al. (1988) conducted acute toxicity tests on 5 fish species from Chesapeake Bay and found 96 h LC_{50} values between 3 – 26.5 μg l^{-1} TBT, while 96 h LC_{50} values of 0.6 μg TBT l^{-1} have been measured in Chinook salmon *Oncorhynchus tshawytscha* which were adapted to seawater (Short and Thrower, 1987). These authors further suggested that TBT exposure might have been the cause of death of salmon exposed to TBT-treated marine net pens in an aquaculture facility at Little Port Walter, Alaska. Similar mortalities, behavioural changes and histopathological effects have been recorded in Atlantic Salmon, *Salmo salar* exposed to TBT-treated pens for 4 days, together with increased TBT concentrations in liver, skin, eyes and other organs of exposed fish (Bruno and Ellis, 1988). Other sublethal effects in fish have been reported, Hall et al. (1984) found behavioural changes in juvenile striped bass *Morone saxatilis* and Atlantic Menhaden *Brevoortia tyrannus* at total organotin concentrations of 24.9 and 5.5 μg l^{-1} respectively. *In vitro* effects of TBT compounds on ionic regulation and gill ATPase activity have been demonstrated in *Morone saxatilis* and *Fundulus heteroclitus* at concentrations between 5.25 – 108 μg l^{-1} TBT (Pinkney et al., 1989). Three-spined sticklebacks, *Gasterosteus aculeatus* exposed to TBTO in seawater at concentrations up to 10 μg l^{-1} TBT suffered from diminished appetite, lethargy and opaque eyes after 4 weeks at the highest concentrations

(Holm et al., 1991). Gonad Somatic Index was reduced in all exposed fish, relative to controls, and fish exposed to 10 μg l^{-1} TBT for 2 months showed cell degeneration and deformation in gill tissues together with other histopathological effects.

Thus, analysis of available data shows the effect of dissolved TBT across a wide range of estuarine and marine organisms, from bacteria and phytoplankton to fish. A wide range of toxicological effects have also been reported from lethally toxic doses, growth and reproduction rate changes and deformities to behavioural and avoidance responses and subtle histopathological effects. Many of the acute lethal effects have been reported at concentrations much higher than have been seen in the natural environment, except perhaps near ship repair and cleaning facilities (Table 1.2), such that lethal effects are unlikely to have occurred widely in nature. Many of the chronic, sub-lethal effects however, have been shown to occur at environmentally realistic concentrations, and in several cases these chronic effect studies have been initiated from field observations of deleterious effects. As mentioned briefly earlier, one of the most widespread and sensitive sub-lethal effects of TBT has been it effects on the sexuality of some marine gastropod molluscs.

1.6 Gastropod Imposex

In most prosobranch gastropod molluscs the sexes are separate and do not change during the lifetime of the individual (Fretter and Graham, 1962). However, Blaber, (1970) reported the occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* from Plymouth Sound, UK. Similarly, penis bearing female mud snails *Ilyanassa obsoleta* were reported from Long Island Sound, US (Smith, 1971) who used the term 'imposex' to describe the superimposition of male characters onto unparasitized and parasitized females. Subsequent investigations (Smith, 1980; Smith, 1981a; Smith, 1981b; Smith, 1981c; Smith, 1981d) determined the primary cause of this phenomenon to be exposure to leachates from TBT-based antifouling paints. Further investigations using the European dog-whelk *Nucella lapillus* (Bryan et al., 1987; Bryan et al., 1986) have shown the imposex response of neogastropods to be one of the most sensitive biological indicators of aquatic TBT contamination, being induced by exposure to concentrations below 1.25 ng l^{-1} TBT, and that the degree of imposex expression is TBT-dose dependent.

Briefly, imposex involves the development of a penis and a vas deferens, which in females extends from the penis to the anterior oviduct. At higher TBT concentrations the imposed vas deferens overgrows the female genital papilla so as to block the vulva, thus preventing the passage of egg capsules and effectively sterilising the female. Capsule formation however remains unaffected, and these accumulate, and may eventually rupture the oviduct causing premature death, which may account for the low proportion of females in affected populations. Imposex is irreversible with no major remission evident with a post-exposure decline in TBT body burden. Its intensity is therefore an indicator of past exposure, particularly during juvenile life when the reproductive tract is developing.

Thus the degree of female masculinization may be so extreme that a sex reversal is effected, although female characters of the oviduct may still remain defined. Quantifying the intensity of imposex has been achieved by the use of indices of penis, and vas deferens development, and has been extensively investigated in the dog-whelk *Nucella lapillus*. The relative penis size index (RPSI) compares the cubic length of female penes to those of males in the same population and has been quantitatively related to TBT exposure (Bryan et al., 1986). The RPSI has been shown to be an easily employed biological measure of TBT contamination, which, when plotted over a wide area clearly indicated regions most impacted by TBT (Gibbs et al., 1987). However, penis development on females although an obvious feature, does not alone lead to sterilisation; it is the vas deferens development at higher TBT concentrations which is more relevant in this respect. As a result the Vas Deferens Sequence (VDS) index was suggested (Gibbs et al., 1987) which divided the development of this feature on females into 6 stages dependent upon TBT exposure. Stages 1 – 4 sequentially describe the development of the vas deferens until it is complete, but does not hinder reproduction; while stages 5 and 6 refer to overgrowth and blockage of the vulva, and capsule retention respectively.

Similar masculinization of female gastropods has been reported for a range of neogastropods worldwide although the literature is too extensive to be reviewed here (see (Gibbs and Bryan, 1996) for review). Indices of female penis size and vas deferens development have also been widely applied, though methods have often been modified on account of apparent differences

between genera and species (Foale, 1993; Stewart et al., 1992; Stroben et al., 1992b; Wilson et al., 1993).

Examination of the morphological expression of imposex in *Hinia reticulata* collected from the Brittany and Normandy coasts of France between 1988 and 1991 showed that although male characteristics were imposed upon females in response to TBT contamination of seawater, neither sterilisation or complete sex change occurred (Stroben et al., 1992b). It was also concluded, based upon the way that imposex develops in this species, and the morphology of male and female penes, that the VDS was the most suitable morphological index of TBT contamination, although uncubed RPS (i.e. Relative Penis Length – RPL) could be used as a secondary index in highly polluted areas (Stroben et al., 1992b). Further studies demonstrated no remission of imposex in *Hinia reticulata* maintained under TBT-free conditions for 18 months (Stroben et al., 1992a).

Imposex, and TBT concentrations in tissues of *Hinia reticulata* and seawater, collected from sites around SW England between 1984 and 1993 have been reported (Bryan et al., 1993b). Measurements made prior to the restrictions on TBT usage in 1987 showed that the intensity of imposex was related to TBT burdens in tissues of females and that bioconcentration factors were approximately 30,000 at 25 ng TBT l^{-1} to 75,000 at 2.5 ng TBT l^{-1}, demonstrating *Hinia reticulata* to be a very good bioaccumulator of dissolved TBT. Additionally, penis development in females was found to be initiated at about 2.5 ng TBT l^{-1}, so that in this respect, *Hinia reticulata* appears to be slightly less sensitive to TBT than *Nucella lapillus*. As a result of TBT restrictions it was found that TBT concentrations in tissues decreased between 1987 and 1993 by factors of 5 – 10 times, while population imposex declined only very slowly. It was therefore concluded that *Hinia reticulata* is a useful complement to *Nucella lapillus* as an indicator of water-borne TBT contamination, but when environmental levels are declining, analysis of *Hinia reticulata* tissues provide a better indication of change than measurements of population imposex.

1.7 Sediment Toxicity

In contrast to the extensive investigations of TBT toxicity in water, there have been relatively few studies of the bioavailability and toxic effects of sediment associated TBT. The

bioavailability and toxicity of contaminants in sediments is inherently more complex than for dissolved contaminants, and sediment dwelling biota may be exposed to sediment-bound toxicants by a variety of routes. They may be exposed to overlying water via their respiratory and body surfaces, and if they burrow, may also be exposed to the sediment by direct contact, and by exposure to interstitial water. Deposit-feeding organisms, by definition, will also be exposed by the ingestion of contaminated sediment. A further complication is the variable nature of sediments themselves, and it has frequently been observed that similar concentrations of a chemical (e.g. in µg of chemical per gram of sediment) can exhibit a range of toxicities in different sediment types (DiToro et al., 1991).

Total tin levels in sediments from UK estuaries have been compared with tin burdens in the deposit-feeding clam *Scrobicularia plana* from the same sites, but demonstrated only poor correlation (Langston et al., 1990). However, a significant relationship was found between Sn concentrations in clams and those in 1M-HCl sediment extracts (a surrogate for bioavailable metals). Despite this however, large deviations were noted at some sites, with abnormally high tin burdens in clams from harbours and areas of high boating activity. Furthermore, it was noted that there appeared to be minimal toxic effects in areas where total sediment inorganic tin concentrations were very high (>2500 µg g^{-1} TBT) while repeated observations had shown declining populations in areas of high sediment TBT. In the Solent for example *Scrobicularia plana* had virtually disappeared from areas where sediment-TBT concentrations were >0.75 µg g^{-1} TBT (Langston et al., 1990). Further examination of field data revealed a significant quantitative relationship between the TBT concentrations in sediments and TBT burdens in *Scrobicularia plana* (Langston and Burt, 1991). Laboratory experiments using TBT-spiked sediments and water showed that there was a predominantly particulate component to TBT accumulation in *Scrobicularia plana* and that TBT was bioconcentrated in clam tissues in proportion to the sediment TBT concentrations. Particulate, as opposed to desorbed TBT was the major vector for bioaccumulation, although some TBT was accumulated from water when this was the sole route for uptake. Sediments containing 25 µg g^{-1} TBT were found to be acutely toxic to *Scrobicularia plana*.

Ruiz et al. (1994a) conducted a solid-phase sediment toxicity bioassay with small *Scrobicularia plana* spat (2 - 3 mm in length) using natural sediments from estuaries in the UK and northern

Spain. A sediment contaminated with non-TBT compounds was found to be acutely toxic (80% mortality in 12 days) and caused sediment avoidance responses, while a TBT contaminated sediment (0.68 μg g^{-1}TBT) produced no mortality or behavioural responses in the short-term. However, both growth and burying activity were significantly affected in TBT contaminated sediments compared with controls implying possible long-term ecological effects in the field such as smaller size and increased predation.

Recent investigations (Meador et al., 1997) have focussed on the toxicity of sediment associated TBT to 3 species of infaunal invertebrates: the polychaete *Armandia brevis* (a non-selective deposit feeder); and the amphipods *Rhepoxynius abronius* (a meiofaunal predator) and *Eohaustorius washingtonianus* (a detritivore). In particular, sediment organic carbon and its effects on partitioning and bioaccumulation of TBT by these biota were studied. Artificially composited natural sediments were employed as substrates and organic contents were manipulated by the addition of natural organic detritus (from an aquarium header tank). An organic carbon normalised partition coefficient (K_{oc}) of 25100 was found for the range of sediments used, while studies on the partitioning of TBT to dissolved organic carbon in interstitial waters (K_{doc}) gave a value of 1652 demonstrating that only a small proportion of TBT in interstitial water was sorbed to dissolved organics and the majority was therefore 'free' TBT. By varying sediment organic carbon content and holding grainsize constant, a 4 – 5 fold increase in LC_{50} and tissue accumulation was observed as the sediment organic levels were reduced. LD_{50} values determined in all 3 species were very similar, and significant mortality was observed when tissue concentrations reached 35 – 80 μg g^{-1} TBT. It was proposed from these results that organic carbon association of TBT in sediments strongly influences the bioaccumulation and toxicity of TBT through its regulation of interstitial water TBT concentrations, and that dissolved TBT in these pore waters accounted for almost all of the TBT found in tissues of the test organisms, such that their mode of feeding was not an important factor in the determination of TBT uptake.

Therefore the current knowledge of the nature of sediment-bound TBT bioavailability, toxicity, partitioning and uptake routes, and the effects of sediment parameters upon these factors, appears to be far less well understood than the effects of dissolved TBT in the aquatic phase of

the environment. Hence the importance of this study to increased understanding of the importance of sediment-bound TBT.

1.8 Biology of *Hinia reticulata* and its use as an indicator of TBT

Hinia reticulata (=*Nassarius reticulatus*) is a member of the sub-order Stenoglossa (=Neogastropoda) of the sub-class Prosobranchia. It is commonly found around the low tide mark on rocky shores where sandy or silty substrates are present, and occurs in relatively sheltered places along the European coastline between mid-Norway and the Black Sea (Fretter and Graham, 1962). It is basically marine, but can tolerate brackish water to a certain extent. It is a detritivore, living on dead and decaying flesh and favours locations where these remains accumulate. At low tide *Hinia reticulata* remains buried just below the sediment surface with only the pallial siphon extending above the sediment. As the tide comes in it becomes more active and mobile, gliding over the sediment or ploughing through the surface layer.

1.8.1 Life History and Ecology

The early life history of *Hinia reticulata* collected from Plymouth, UK was reported earlier this century (Lebour, 1931). It was reported that the transparent, horn coloured egg capsules were laid in early summer, although eggs could be found in most months of the year, and that these 4 – 5 mm diameter capsules were frequently attached to the fronds of seagrass, *Zostera marina*. Individual females may lay 8 – 66 (n=35) capsules with 126 – 322 (n=60) eggs per capsule (Tallmark, 1980). Hatching occurs within one month of the capsule being laid (Lebour, 1931) although this has been shown to be temperature dependent (Tallmark, 1980). The newly hatched larva (approximately 0.3 mm across) has a smooth unsculptured shell and a bilobed ciliated velum. At this stage the planktonic larvae feed on other plankton by ciliary action and are most common at depths of 5 – 10 m (Fretter and Shale, 1973). After 25 days the larva is about 1.3 mm across with a shell comprising 2 – 3 whorls, and the velum becomes 4-lobed. At about 6 weeks the foot has begun to form, together with tentacles, and as the anterior end of the foot expands the animal is able to crawl. It continues to crawl or swim at will, but the velum dwindles and disappears once the animal finds a suitable substrate. It has been shown that the American mud-snail, *Nassarius obsoletus* was capable of temporarily delaying metamorphosis until a suitable substrate is found, thus increasing its chances of survival (Scheltema, 1961).

Detailed investigations of the population dynamics of *Hinia reticulata* in Gullmar Fjord, Sweden, between 1973 and 1979 demonstrated the settling of veliger larvae to be very irregular, and that it normally only occurred where the organic content of the substrate was fairly high (Tallmark, 1980). Small *Hinia reticulata* (<15 mm) were found to be greatly attracted to detritus-rich substrates and spent the first three years living in the settlement area and were assumed to be living primarily upon detritus (Tallmark, 1980). Larger individuals were found to be capable of surviving on detritus alone and could survive starvation for 5 months, but preferred larger carrion (e.g. dead *Cerastoderma edule*) as a main food source, which was shown to be essential for growth and fecundity of larger individuals. *Hinia reticulata* in this population were found to mature at about 4 years old (approximately 15 mm) and from then on most of them participated in annual migrations, moving offshore to deeper water in winter and back to shallower, inshore water in summer (Tallmark, 1980). Growth was found to be rapid in the first 3 years but then decreased considerably and showed greater individual variability. Maximum age was estimated to be about 15 years. Fecundity was high: about 6000 eggs female^{-1} year^{-1} and females spawned every year. Mortality was high during the planktonic phase but there were few predators on larger whelks, although trematode parasitism was suggested as an important cause of losses at that stage since parasitized individuals were found to be less resistant to temperature and salinity stress, and did not migrate, therefore becoming prone to seasonal temperature changes. Moreover, infected whelks became sterile, which is believed to be due to the secretion of endocrine disrupting metabolites by the parasites (Feral et al., 1972), such that avoidance of parasitism was of primary survival importance.

The influence of environmental factors on the diurnal rhythm of *Hinia reticulata* from a non-tidal area (Gullmar Fjord, Sweden) has shown that natural photoperiod and temperature regulate the rhythm and activity level, and that in summer the diurnal rhythm is controlled by light so that the species is active by night, and inactive, buried in sediment during the day (Eriksson and Tallmark, 1974). It was suggested that in common with other species showing similar rhythms this imparts a survival advantage by avoiding predation from sea birds and fish. In winter however, at 5°C or below, the rhythm disappears, while field observations showed that the animals moved further offshore to deeper water thus avoiding exposure to freezing temperatures. Experiments on temperature-salinity tolerance (Eriksson and Tallmark, 1974)

showed *Hinia reticulata* to be markedly eurythermal and capable of withstanding 96 h within the temperature interval of 0 – 25°C at normal salinity (25‰). However they become more salinity sensitive at higher temperatures and could only withstand 20 - 30‰ at 25°C, but could cope with 10 - 40‰ at 5°C, such that *Hinia reticulata* has a combined dependence on temperature and salinity and is well adapted to its habitat, characterised by strongly varying physical parameters. Further experiments on substrate preference (Eriksson et al., 1975) showed that *Hinia reticulata* prefers sandy substrates to rock, cobbles or pebbles, and that sandy substrates with a high detrital content were preferred over clean sand.

1.8.2 *Hinia reticulata* as an indicator of TBT pollution

Soon after the first report of penis-bearing female *Nucella lapillus* (Blaber, 1970) a similar phenomenon was reported in the American mud snail *Nassarius* (=*Ilyanassa*) *obsoletus* and termed 'imposex' (Smith, 1971). Further investigations (Smith, 1980; Smith, 1981a; Smith, 1981b; Smith, 1981c; Smith, 1981d) were among the first to link the imposex phenomenon with TBT, and set the scene for studies worldwide. Thus, nassariids have been studied in connection with TBT pollution almost since the beginning of interest in TBT ecotoxicology. During the development of *Nucella lapillus* as an indicator for TBT contamination in the UK, it was apparent that its use would be restricted as terminally affected populations died out and that another, perhaps less sensitive indicator species would be useful (Bryan et al., 1993b). An alternative candidate appeared to be the netted dogwhelk *Hinia reticulata* which occurs on sediment shores, overlapping to some extent with *Nucella lapillus* and therefore providing a complementary role in TBT studies (Bryan et al., 1993b). An additional advantage in the use of *Hinia reticulata* is that this species is not sterilised like *Nucella lapillus*; vas deferens tissue does not block the vulva, while a relatively long planktonic stage in the life cycle allows for recruitment into affected areas. The effects of TBT on *Hinia reticulata*, its morphological expression of imposex and its use as a bioindicator of TBT in comparison to *Nucella lapillus* have been studied recently (Bryan et al., 1993b; Stroben et al., 1992a; Stroben et al., 1992b). As a brief summary: Bryan et al. (1993b) found no significant difference between TBT and DBT concentrations in male and female *Hinia reticulata*, although (Stroben et al., 1992b) working on animals from northern France found lower levels (89% and 86% respectively) in males compared with females. The relationship between TBT concentrations in tissues and those in

seawater showed concentration factors from 28,500 at 25 ng l^{-1} TBT to 74,500 at 2.5 ng l^{-1} TBT (Bryan et al., 1993b) and a regression between tissue and water concentrations showed significant tissue burdens at zero TBT in water, implying a proportion of this is derived from other sources (food, sediment (Stroben et al., 1992a)). Comparisons between *Hinia reticulata* and *Nucella lapillus*, including indices of imposex and tissue accumulation showed the vas deferens sequence (VDS) to be the most suitable index of effect, followed by relative penis length (RPL). However, since imposex is irreversible, and population imposex declines only slowly due to the longevity of this species, analysis of *Hinia reticulata* tissues provide a better indication of impact when environmental TBT levels are declining (Bryan et al., 1993b).

1.9 Rationale and Aims of this thesis

From this review of current knowledge on the occurrence and effects of TBT, it is apparent that TBT in the water column has received much greater attention than sediment-associated TBT. Current regulations and Environmental Quality Standards (EQS) refer to water column data alone, there being no EQS for sediments, reflecting the paucity of information on bioavailability and effects of sediment-bound TBT.

However, the sorption of dissolved TBT onto particulates and its incorporation into sediments, together with slower degradation, and greater persistence of TBT in sediments means that sediment-bound TBT may remain as an environmental concern long after concentrations in the overlying waters have decreased.

The research described within this thesis has investigated several important aspects of sediment-bound TBT:

- The sorptive behaviour of TBT has been extensively studied in order to understand the effects of time, TBT concentration, suspended sediment load, pH and salinity on the distribution of TBT between sediments and water under near-natural conditions. In doing so, some of the uncertainty and contradiction over certain aspects of TBT sorption reported in other investigations has been removed.
- Studies of TBT equilibrium partition coefficients have been conducted across a range of natural sediments, and related to their physicochemical parameters to try and determine

the type(s) of sediment which may pose the greatest risk as long-term reservoirs, and secondary sources of TBT.

- Long-term laboratory studies of sediment-bound TBT have determined the potential for re-release of sedimentary TBT to overlying waters, together with estimation of TBT degradation rates in sediments and release of degradation products. Limited data on the effects of bioturbation on TBT release from sediments are also presented.
- The bioavailability of sediment-bound TBT has been investigated using the sediment dwelling gastropod *Hinia reticulata*. Uptake and depuration of ^{14}C-labelled TBT by this sensitive species has been studied together with its quantitative response to dissolved and sediment-bound TBT concentrations. The relative importance of these uptake routes has been estimated together with tissue distribution and metabolism of TBT in *Hinia reticulata*.
- With its quantitative responses and uptake routes established, *Hinia reticulata* has been employed as a test organism to estimate the bioavailability of TBT from a range of natural sediment types, previously investigated as part of the equilibrium partitioning studies, to extend information on the types of sediment which may pose the greatest risk as TBT sources.

Therefore, studies described within this thesis have extended current knowledge on the behaviour and importance of sediment-bound TBT.

2 MATERIALS AND GENERAL METHODS

Some general methods, which have been used throughout this work, are described here to avoid repetition. These protocols were developed as an integral part of this research programme and represent robust and quantitative methods for the extraction and analysis of ^{14}C-TBT in seawater, sediments and biological tissues. Where other, more specific methods have been used, details are given in the relevant chapters.

2.1 ^{14}C-Tributyltin

This research programme has been designed with ^{14}C-labelled tributyltin chloride as the TBT source in all experimental procedures. The use of a labelled compound confers several advantages over unlabelled compounds, which have more often been employed in studies on TBT. Principally, the relatively high specific activity of the labelled compound, combined with detection by Liquid Scintillation Counting (LSC) allows analysis at very low TBT concentrations to be carried out with greater accuracy and precision than could be achieved with an unlabelled TBT compound, thus simplifying extraction and pre-concentration techniques.

Specifically, the compound employed throughout this research was [Tri-n-(1-^{14}C) butyl tin chloride] supplied to the Plymouth Marine Laboratory by Amersham International in October 1987, with a radiochemical purity of 95% (November 1986) and a specific activity of 21 mCi/mM (non-SI units used at that time). 10 mCi were purchased which should have equated to 155 mg of the solid compound. However, only 150 mg were present leading to some slight doubt about the specific activity. Nevertheless, this solid ^{14}C-TBTCl compound was dissolved in absolute ethanol and made up into a series of working standards as follows:

Standard 1.

150 mg TBTCl in 10 ml ethanol equivalent to:

15 mg ml^{-1} or approximately 1 mCi ml^{-1}

Standard 2.

A 100-fold dilution of Standard 1 with absolute ethanol equivalent to:

0.15 mg ml^{-1} or approximately 10 µCi ml^{-1}

Standard 3.

A 50-fold dilution of Standard 2 with absolute ethanol equivalent to:

0.003 mg ml^{-1} or approximately 0.2 µCi ml^{-1}

All standards were made up in glass volumetric flasks and stored at -20°C in the PML radioactive isotope store. Standard 1 was retained within lead shielding due to significant levels of *bremsstrahlung* radiation resulting from high activity of a ß-emitter contained in a dense material (glass).

2.2 Spiking of sediments with ^{14}C-TBT

Sediments were collected by hand from a range of locations in southern England, summarised in the next chapter (Figure 3.19). All sediments used in experimental procedures were kept in as near to field condition as possible. After collection, these aerobic surface sediments were sieved with seawater through a 1mm mesh nylon sieve to remove large detrital particles (leaves, twigs etc.) together with large macrofauna. The sieved sediment suspension was then allowed to settle and the supernatant water decanted off, leaving a sediment slurry. This slurry was then mixed using an electric benchtop mixer with PTFE coated paddle. Aliquots of slurry were then taken and dried to determine a wet/dry weight ratio for the sediment slurry in question. Once this was determined, a wet mass of slurry, equivalent to the dry weight required, was weighed out into a polythene bucket and stirred with the mixer. Additional water was added to adjust the slurry to a suitable consistency for mixing, and the required amount of ^{14}C-TBT in ethanol was added using a Finnpipette with disposable tips. The spiked sediment slurry was then mixed thoroughly for at least 10 minutes, covered and allowed to stand overnight at 15°C. Supernatant water was removed and approximately 1 litre of seawater was then added and the sediment thoroughly mixed again, and allowed to settle overnight once more. Once settled, the sediment was transferred by washing into the experimental tank and allowed to settle.

2.3 Extraction and Analysis of Seawater

Water samples (0.5 L) were acidified with 5 ml acetic acid (99.8%, Aldrich) on collection and extracted immediately by transfer to a 1 L glass separatory funnel, followed by addition of 5 ml

toluene (99%, Aldrich). This mixture was shaken vigorously for 4 minutes and then allowed to stand until the organic and aqueous layers had separated, the organic layer being collected into a 10 ml glass centrifuge tube. A 1 ml aliquot of this toluene extract was pipetted into a 20 ml liquid scintillation vial containing 14 ml of Optiphase 'MP' liquid scintillation cocktail (Pharmacia - LKB) and the activity determined using an LKB - Wallac 1215 Rackbeta II liquid scintillation counter. The remainder of the toluene extract was shaken for 1 minute with an equal volume of 1N sodium hydroxide to remove any DBT present, centrifuged, and a further 1 ml aliquot of this washed extract taken for scintillation counting as before.

Recoveries and Precision

Prior to its use in experimental protocols, the extraction efficiency and precision of this method was tested using batches of seawater that had been dosed with a known amount of ^{14}C-TBTCl. The results for 4 replicate analyses of water samples spiked with TBTCl are shown in Table 2.1 and demonstrate a mean recovery of 99.1% ± 2.4%.

Table 2.1 Recovery of ^{14}C-TBT from spiked seawater.

TBT added (ng)	TBT recovered (ng)	Recovery (%)
146.0	143.5	98.3
146.0	149.8	102.6
146.0	142.1	97.3
146.0	143.2	98.2
		Mean = 99.1 ± 2.4

2.4 Extraction and analysis of sediments

Samples of wet sediment slurry (1.0 ml) were pipetted using wide bore pipette tips; into pre-weighed 30 ml stoppered glass centrifuge tubes and reweighed. In each case, a sediment sub-sample was taken for oven drying to determine the wet to dry weight ratio of the slurry. 10 ml acetic acid (99.8%, Aldrich) was added to each sample tube and the contents mixed on a vortex mixer and allowed to stand overnight. 5 ml toluene (99%, Aldrich) was then added and the stoppered tubes shaken vigorously for 15 minutes on a bench 'wrist action' shaker. After shaking, 10 ml of distilled water was added to each tube and mixed thoroughly prior to

centrifuging at 3000 rpm for 5 minutes. On completion, the organic layers were removed by pipette from each tube into smaller centrifuge tubes. A second extraction was then completed as above, with a second 5 ml aliquot of toluene. In each case, 1 ml aliquots of each extract were pipetted into 20 ml glass liquid scintillation vials containing 14 ml Optiphase 'MP' cocktail and the activity determined on an LKB Rackbeta II scintillation counter. The remainder of each of the first and second extracts were shaken with equal volumes of 1N sodium hydroxide to remove any DBT present, centrifuged, and further 1 ml aliquots of these washed extracts taken for scintillation counting as before.

Recoveries and Precision

The protocol described above, when used on sediment samples which had been spiked with 60 ng of ^{14}C-TBTCl gave a mean recovery of 95.7% ± 2.4% (n =4), with 90.5% being recovered in the first extract alone (Table 2.2).

Table 2.2 Recovery of ^{14}C-TBT from spiked sediment.

TBT added (ng)	TBT recovered in first extraction (ng)	TBT recovered in second extraction (ng)	Total recovery (%)
60.0	54.75	3.22	96.6
60.0	55.79	3.24	98.4
60.0	53.86	3.22	95.1
60.0	52.82	2.85	92.8
			Mean = 95.7 ± 2.4

2.5 Extraction and analysis of *Hinia reticulata* tissues

Methods used for the extraction of TBT from tissues follow those used previously at PML (Bryan et al., 1993a). Individual *Hinia reticulata* once removed from the experimental system were allowed to depurate in clean seawater for 2 – 3 days to remove adhering sediment particles. Individual animals were then cracked in a plastic coated bench vice and the soft tissues either frozen whole in pre-weighed glass vials, or dissected and pooled tissues similarly frozen, depending on the experimental detail. Tissues were frozen for a maximum of one month prior to extraction and analysis. As an example, a whole *Hinia reticulata* (soft tissue) was thawed and homogenised in 5 ml of distilled water using a small stainless steel homogeniser

(Ultra Turrax). Of this homogenate one 1 ml aliquot was then dissolved in 1 ml of Optisolv tissue solubilizer (Pharmacia - LKB) in a liquid scintillation vial. Tissue dissolution was usually complete in 2 – 3 days, and once dissolved 14 ml of Optiphase MP scintillant was added and activity determined on an LKB Rackbeta II scintillation counter to give the total ^{14}C activity in the tissue (i.e. TBT, DBT and other metabolites). A second aliquot of homogenate (1 – 2 ml depending on anticipated TBT levels) was pipetted into a pre-weighed 30 ml glass-stoppered centrifuge tube, reweighed, and 5 ml of concentrated hydrochloric acid (Fisons AR grade) added. This was then extracted with 5 ml of hexane by shaking for 15 minutes on a bench wrist-action shaker. Of this extract 1 ml was counted for ^{14}C (TBT and DBT fraction) and the remainder (TBT fraction) was counted after shaking with an equal volume of 1M sodium hydroxide to remove DBT. Remaining unused homogenate was oven dried to obtain a wet to dry weight conversion factor for the tissue. All results are presented on a dry weight basis unless stated otherwise.

Earlier studies on dog-whelk tissues which had been spiked with organotin compounds showed that these procedures will largely separate TBT and DBT from each other and from MBT (Bryan et al., 1986) but it has been stressed (Bryan et al., 1993a) that these relatively simple methods do not provide complete speciation of TBT and its metabolites. In addition to TBT and DBT, the 'total' (Optisolv) extract could contain other labelled compounds arising from TBT breakdown, for example MBT and hydroxybutyldibutyltin (Lee, 1985). Furthermore, the tri-n-(1-^{14}C) butyltin chloride used in this study contained three ^{14}C atoms per molecule (assuming complete and uniform labelling) on each butyl group. Therefore during debutylation of TBT to DBT, MBT and other intermediate metabolites such as hydroxydibutyltins the specific activity changes along with the molecular weight of the compound to which the ^{14}C is attached. Since the precise speciation of compounds present in the 'total' extract are uncertain (other than TBT and DBT) these results have been presented as an equivalent mass of TBT (and in many instances the mass of TBT determined from alkaline washed hexane extraction was very similar). However, it must be stressed that where the proportion of TBT in the 'total' is low, the uncertainty in composure of the 'total' increases. Nevertheless it was felt that presentation in this form was perhaps more meaningful in the context of the majority of published data, rather than in units such as DPM g^{-1} although the latter, strictly, would be more correct. In retrospect, it would have

been very advantageous to have had a TBT substrate labelled at the Sn atom rather than on each butyl group since the activity of a molecule through the series TBT, DBT, MBT and any intermediates would be the same and would have allowed more accurate direct comparisons of extracts.

Therefore only the values presented for TBT may be described as accurate, those for 'total' and 'TBT+DBT' are approximate, but nevertheless, provide a useful insight into the degradation and metabolism of TBT.

2.6 Liquid scintillation counting

Analyses of all ^{14}C-labelled organotin extracts were performed using an LKB-Wallac 1215 Rackbeta II liquid scintillation counter. Optiphase MP proved to be the most suitable liquid scintillation cocktail despite its xylene solvent base being potentially more hazardous than other 'safe' scintillants. Two quench curves were prepared for use with Optisolv tissue solubilizer extracts, and toluene (or hexane) extracts respectively.

Since there had been an element of uncertainty over the specific activity of the ^{14}C-TBTCl it was decided to prepare a series of reference standards to be counted along with all samples so that calibration could be effected with respect to measured values rather than an uncertain prescribed value. Thus, 4 replicates of each of the following standards were prepared and counted with each analysis:

150 ng TBTCl in ethanol added to 14 ml Optiphase MP (Total activity).

150 ng TBTCl in ethanol added to toluene, washed with distilled water to remove ethanol and added to 14 ml Optiphase MP (TBT + DBT fraction).

150 ng TBTCl in ethanol added to toluene, washed with 1M NaOH to remove any DBT and added to 14 ml Optiphase MP (TBT fraction).

The use of these standards showed the stock of TBTCl to be of >95% purity as supplied, and, furthermore it inherently added a temperature correction to all analyses since examination of standard counts showed some significant variations with ambient temperature (the efficiency of the scintillation process being temperature dependent). Therefore the analyses conducted in

this investigation have been executed with a greater degree of certainty than if the suppliers prescribed specific activity alone had been used.

2.7 Data processing and statistical procedures

All data were compiled into *Microsoft Excel* spreadsheets allowing simple data management, calculation, processing and graphic output. Statistical analyses were performed using the *Statistica* software package (Statsoft Corp.). All statistical analyses were performed using parametric methods since these are more powerful than non-parametric methods when used correctly (Zar, 1984). Parametric testing assumes that the data agrees with a number of criteria. The most important of these assumptions is that the data are normally distributed and that they have homogeneous variances. Although analysis of variance is relatively robust to deviations from these assumptions all data were tested for normality using either the Kolmogorov-Smirnov test or Shapiro-Wilks' W test; and for homogeneity of variances using the F-max test recommended in the *Statistica* package. Where necessary, data was log transformed and re-tested.

2.8 Collection of Hinia reticulata

Hinia reticulata used in all procedures were collected by hand from Talland Bay (O.S. grid ref.: SX250510) on the south Cornwall coast. Talland Bay is a small sandy bay, which slopes gently offshore, providing extensive areas of clean sub-tidal sand. In the centre of the bay the rocky shoreline extends as a wave-cut platform of reefs, criss-crossed with sandy gullies and shallow sandy pools. These gullies and pools support a large population of *Hinia reticulata,* which appear to thrive on the abundant detrital matter present on the sediment surface. The most effective method of collection was found to be by baiting pools with crushed mussels, *Mytilus edulis* and waiting a few minutes for *Hinia* to become active and move towards the bait. One person could easily collect over 300 individuals over a low tide period by this method. For consistency, only larger individuals (>20 mm total shell length) were used in experiments, smaller individuals being rejected on site. All organisms collected were transported live to the laboratory and maintained in clean flowing seawater for a few days, until introduced into the respective experimental system.

3 SEDIMENT-WATER PARTITIONING OF TBT

3.1 Introduction

3.1.1 Sediment-water partitioning and bioavailability

In assessing the bioavailability of sediment-bound toxicants, consideration must be given to all possible avenues of exposure (Anderson et al., 1987). Organisms in the water column are exposed primarily to toxicants released from the sediment back into the overlying water. Benthic species however may be exposed via two additional routes:

1. The ingestion of contaminated sediment particles.
2. Exposure to toxicants in the sediment interstitial water e.g. across respiratory surfaces, and the body wall.

The sequestration of TBT by sediment particles together with its subsequent sorption behaviour is therefore of paramount importance when attempting to elucidate the relative importance of these avenues of exposure for benthic organisms (Anderson et al., 1987).

Sorption is used as a general term, encompassing both adsorption and absorption, where *adsorption* is used to describe that portion of a compound binding to sediment particle surfaces, which is readily reversible, i.e. described by surface complexation. *Absorption* describes that portion of a sorbed compound which possesses a significant time dependency, resulting from its diffusion into the pores of aggregates within the solid phase (Jenne and Zachara, 1987).

The sediment-water partitioning approach is complicated by the fact that sediments differ greatly in their physicochemical parameters, and that sediment distribution in estuaries is largely dependent upon the hydrodynamic energy of the environment, together with the availability of component materials. On a smaller scale, individual sediments are not homogeneous phases, but are in fact highly composited mixtures of components, including mineral particles, organic materials and amorphous oxide layers. Each of these components may be presumed to differ in their thermodynamic affinity for TBT; their availability for sorption; and the nature of their sorption process (binding mechanism, site specificity, etc.). Sediment

heterogeneity at these meso- and micro-scales means that sorption behaviour may warrant site-specific investigation, and that generalisations may not be possible.

This study has investigated sediment-water partitioning of TBT, with the aim of using the knowledge gained to assist in assessment of the relative importance of different routes of sediment-bound TBT exposure to benthic organisms; in particular the gastropod *Hinia reticulata*.

3.1.2 The Partition Coefficient

There have been some excellent reviews in recent years of the theory and background of metal and hydrophobic compound sorption by sediments (Jenne and Zachara, 1987; Voice and Weber, 1983; Weber et al., 1983).

The simplest model is that of Linear Sorption or Constant Partitioning where an equilibrium partition coefficient (K_d) expresses the relationship between the concentration of a component attached to the sediment phase (C_s), and the concentration in the surrounding water (C_w) when the two are in thermodynamic equilibrium.

$$K_d = \frac{C_{sed}}{C_{water}}$$

More complex models include the Langmuir model, developed from the adsorption of gases onto solids, and assumes: constant sorption energy; sorption only on localised sites with no interaction between sorbed molecules; and most importantly, that the maximum sorption possible is that of a complete monolayer. This model was extended to include multi-molecular layers – the BET model, while the Gibbs model considers changes in surface concentrations [Voice, 1983]. However, these complex models have often been found to be inadequate descriptors of sorption data. (Freundlich, 1926) found sorption data to be better described as:

$$C_{sed} = K_F * C_{water}^{1/n}$$

where K_F and n are constants. This equation linearizes in logarithmic form to:

$$\ln C_{sed} = \ln K_F + (1/n * \ln C_{water})$$

Freundlich, (1926) attempted to attach physical significance to the constants K_F and *n* but was largely unsuccessful. The value of K_F however can be taken as a relative indicator of the sorption capacity, while $1/n$ is indicative of the intensity of the reaction (Weber, 1972). More recently a Distributed Reactivity Model (DRM) has been proposed (Weber et al., 1992) comprising linear and non-linear components to characterise the intrinsic heterogeneities in the properties of sediments and to capture the resulting non-linearities of sorption isotherms.

However, all these models reduce directly or indirectly to the linear model under certain conditions, notably they all tend to predict linear sorption at low solution concentrations (Voice and Weber, 1983; Weber, 1972). This is an important factor to be taken into account when considering some of the values for TBT partition coefficients that have previously been reported.

Thus, high values for K_d represent high affinity of the sediment phase for the component, and low K_d values describe the reverse. Clearly, factors that influence the partitioning behaviour of a compound will affect its relative abundance in each phase, and may subsequently affect its bioavailability.

In practice, sorption studies have been conducted by equilibrating known quantities of a sorbent with a solution of the compound of interest. By plotting the variation of solid-phase concentration versus solution-phase concentration at equilibrium a sorption *isotherm* is created, and the models described have been developed to explain these relationships.

Measurements of the concentrations of a contaminant in field samples of water and suspended sediment will yield an apparent partitioning or sorption coefficient, which will approach the equilibrium K_d value only in cases where the sediments are in equilibrium with the overlying water.

TBT has a relatively low aqueous solubility, 0.75 mg l^{-1}, (Maguire, *et al.*, 1983), and an octanol/water partition coefficient (K_{ow}) of 5500 - 7000 (Laughlin, *et al.*, 1986). In common with similar hydrophobic compounds it is readily scavenged by particulate matter, but measurements of apparent K_d values for TBT in field samples (Unger et al., 1988; Valkirs et al., 1986a) have shown considerable variation, which may be partly related to the variable organic

Table 3.1 Reported sediment-water partition coefficients for TBT

Partition coefficient ($l\ kg^{-1}$)	Suspended sediment load ($mg\ l^{-1}$)	Salinity ($g\ l^{-1}$)	pH	Notes	Reference
3278 – 3918 929 340	5.8 - 6.7 14 50	sea water sea water sea water			(Valkirs et al., 1986a)
3000	10	sea water			M & T Chemicals (unpublished). Referred to in (Valkirs et al., 1986a).
1500 - 1900000	10 - 1000	Artificial sea water	6.2 - 8.2	Fulvic acid coated hydrous iron oxide used as the solid phase.	(Randall and Weber, 1986).
2180	5000	sea water			(Maguire and Tkacz, 1985).
111 - 8200	no data	0 – 35 (Artificial seawater)		Only aqueous phase analysed.	(Unger et al., 1988).
6250 - 55439	no data	sea water		*In situ* measurements in Pearl Harbor mesocosms.	(Stang and Seligman, 1987).
4608 - 39352	1.6 - 8.6	sea water			(Valkirs et al., 1987b).
200 - 1400	60 - 100	0 – 32 (filtered seawater)		Sediments were freeze dried, ground, and sieved.	(Harris and Cleary, 1987).
17 – 4500 32 - 292000	1000	seawater		Experimentally determined values. Calculated from field analyses of sediments and overlying water.	(Kram et al., 1989)

content of sediment. For the partitioning of hydrophobic compounds it may be more appropriate to report K_{oc} values where the concentration of the contaminant in the sediment is normalised to the organic content (DiToro et al., 1991). However, the binding of a polar molecule such as TBT may be a function of its hydrophobicity, associated with the large butyl groups; and its polarity, so that the organic content of the sediment alone may not control adsorption. Sediment particle size (or more specifically surface area) is also likely to be important, together with other factors such as the presence of amorphous oxide coatings on particles, salinity and pH. Finally, equilibrium partition coefficients will only be applicable in dynamic environments such as estuaries if the physical and chemical partitioning processes are not limited kinetically, although previous studies with TBT have shown equilibration to be relatively fast (Harris and Cleary, 1987). There have been several investigations of sediment-water partitioning of TBT, and the results of these are summarised in Table 3.1. However, these studies may have limited environmental significance due to the procedures used in their determination. Ideally, in sorption studies, concentrations in both phases should be determined analytically. For several of the studies reported in Table 3.1 this was not the case, and only one phase was analysed, the concentration in the other being calculated by mass balance. In addition, sediments used were often pre-treated, e.g. air or freeze-dried, ground, or sieved; factors which (Podoll and Mabey, 1987) stress should not be applied in such investigations. Finally, the TBT concentrations used in some of these partitioning studies have very often exceeded environmental levels by several orders of magnitude. Consequently the extrapolation of such results to the environment may be misleading, given the complex nature of sorption isotherms and their non-linearity at higher concentrations.

Throughout the investigations conducted in this study emphasis has been placed upon:

1. Robust and quantitative laboratory protocols utilising ^{14}C labelled TBT with analysis in both phases.
2. The use of substrates in conditions as similar to those found in the environment as possible, i.e. freshly filtered sea water, and where required river water; and fresh sediment in as near to field condition as practical.

3. Concentrations of TBT in each phase which approximate to those found in UK estuarine environments.

Within these experimental constraints the effect of several factors on sediment-water partitioning of TBT have been investigated. These include: time; TBT concentration; suspended sediment load; salinity; pH; and sediment type (including physicochemical factors such as grainsize distribution, organic content, humic acid content, and Fe and Mn oxyhydroxides).

In addition, experimental studies have been conducted on the desorption of TBT from contaminated sediments, in the short-term, and over a period of almost 2 years, in order to further assess the long-term potential of such sediments to act as sources of TBT to overlying water.

3.2 Materials and Methods

3.2.1 Experimental determination of the sediment-water partition coefficient (K_d)

This method is a general outline of the protocol developed for the determination of the sediment-water partitioning behaviour of tributyltin under laboratory conditions. It formed the basis of most of the experiments described subsequently, although these often involved modifications depending upon the aims of the specific investigation.

In these experiments 2 - 5 replicates were performed at each experimental level or at each treatment. This section describes the method as conducted for each replicate.

A sample of sediment slurry was obtained and approximately 1 ml weighed into a plastic weighing boat. A second sediment sample was taken to determine the wet/dry weight ratio of the slurry so that results could be expressed on a dry weight basis. Washing transferred the wet sediment slurry from the weighing boat into a clean; acid washed 1-litre glass stoppered conical flask, using 500 ml of freshly filtered (0.45 µm) 35‰ seawater. Each flask was spiked with a known amount of ^{14}C-TBT, usually 150 ng (0.37 kBq), and then mounted on an orbital shaker table set at 250 RPM for 24 hours in the dark at 15°C.

At the end of the shaking period the aqueous and solid phases were separated using Millipore vacuum filtration apparatus with 0.45 µm nylon membrane filters (Schleicher & Schuell). Each flask was rinsed twice with 20 ml distilled water to remove any remaining sediment particles,

and the walls of the filter funnel were washed with a small volume of distilled water to ensure that all solid material was collected on the filter membrane.

The filtrate was immediately acidified with 5 ml acetic acid and extracted by the method described earlier (Chapter 2), to determine the concentration of TBT in the sea water phase.

The sediment, together with the filter was carefully transferred to a 30 ml stoppered centrifuge tube, 10 ml acetic acid added, and allowed to stand overnight prior to extraction as described earlier (Chapter 2) to determine the TBT concentration in sediment.
Previous experiments had shown there to be negligible adsorption of TBT by the filter membrane alone.

Within the protocol described, there is the possibility that some TBT may become adsorbed to the sides of the flasks during the course of the experiment. To determine the magnitude of this 'loss' each flask when empty was rinsed with 250 ml distilled water. 20 ml acetic acid was then added and the flasks shaken before being filled with distilled water and left to leach overnight. The contents were then extracted using the method described for seawater. Provided that all flasks were acid washed prior to use, adsorptive losses to the glass were negligible.

3.2.2 Sediment Grainsize Analysis

Methods used for the analysis of sediment particle size closely followed British Standard methods (B.S.I., 1975). Two methods were employed: wet sieving was used for coarse sediments; and Andreasen's pipette method for fine-grained sediments.

3.2.2.1 Wet Sieving

About 100 g of wet sediment was weighed into a 1 litre plastic beaker, covered with sodium hexametaphosphate solution (33g $Na(PO_4)_6$ + 7g Na_2CO_3 per litre) and stirred well to thoroughly mix the sediment. This was allowed to stand for at least 1 hour, with frequent stirring.

The sediment was then washed, a little at a time, through nested 2mm and 63 µm test sieves (Gallenkamp) until the water passing the 63 µm sieve was clear. When the whole of the sample had been washed, the retained material was dried in an oven at 105 - 110°C.

When dry, the material was sieved through nested 600 µm (coarse sand), 212 µm (medium sand) and 63 µm (fine sand) standard sieves and the weight of material retained on each sieve recorded and calculated as a proportion of the total.

3.2.2.2 Sediment Particle Size Analysis - Andreasen's Pipette Method

Pre-treatment

50 – 60 g of wet sediment slurry was weighed into a 500 ml wide-necked conical flask.

50 ml distilled water was added, mixed thoroughly and gently boiled on a hotplate until the volume was reduced to approximately 40 ml.

75 ml hydrogen peroxide was added in 5 ml aliquots (taking care to avoid excessive foaming), and allowed to stand overnight.

This mixture was heated gently with mixing, and boiled until the volume was reduced to approximately 50 ml. It was then transferred to a pre-weighed 250 ml tall-form beaker, taking care not to lose any sediment, and made up to 200 ml with distilled water.

This was allowed to settle for at least 24 hrs (preferably over a weekend), the supernatant decanted off, and the remaining sediment dried in an oven at 110°C prior to weighing.

Dispersion

100 ml of distilled water was added to the dry sediment in the beaker, allowed to soak, and stirred thoroughly.

25 ml of Sodium hexametaphosphate solution (33 g $Na(PO_4)_6$ + 7g Na_2CO_3 per litre) was added next and mixed on a magnetic stirrer for 4 hours.

The dispersed sediment was then washed over stacked 600, 212, and 63 µm mesh sieves with distilled water. That which passes through the sieves being funnelled into a graduated settlement cylinder.

Sedimentation

An Andreasen cylinder was filled to the 20 cm mark, the appropriate pipette inserted, and the contents thoroughly mixed by inverting the entire apparatus several times.

The cylinder was placed on a bench and the first sample immediately withdrawn (t=0) over a period of 30 seconds. The sample, together with any washings was transferred to a pre-weighed vial.

Further samples were taken at 5, 15, 45, 150, 450 and 1380-minute intervals and dried to determine the mass of sediment removed at each time.

To enable a correction to be made for the mass of dispersant present in the dry sediment samples, a separate cylinder containing 25 ml of the Sodium hexametaphosphate solution diluted to 500 ml, was sampled as described above and the dry weight of dispersant subtracted from the weight of the sediment.

3.2.3 Sediment Organic Content

The organic content of sediments was determined by weight loss on ignition (Bryan et al., 1985). Dry sediment samples were ground with a ceramic mortar and pestle, and a 1 – 2 g sample placed in a ceramic crucible which was dried in an oven at 80°C, and cooled in a desiccator for 1 h prior to weighing. Dried samples were then transferred to a muffle furnace and heated to 400°C for 6 h, cooled in a dessicator and re-weighed. All results were corrected for the loss of seawater salts at 400°C.

3.2.4 Sediment humic acid content

Humic acid coatings on sediment particles are known to be important in the sorption of metals and hydrophobic organic compounds. The nature of humic substances is to a certain extent undefined, and methods used in their quantification are usually operationally defined. The method used in this study, involved extraction of humics into 0.1M NaOH and measurement of the absorbance of this extract at 460 nm (Luoma and Bryan, 1981).

Briefly, 4 ml of sediment slurry was pipetted into a pre-weighed 50 ml conical flask and reweighed. A second aliquot of sediment slurry was dried to determine the dry weight, corrected for salt content. 40 ml of 0.1M NaOH (*Aristar* – Merck) was added to each flask and left to extract, with intermittent agitation, for 1 week. Extracts were then decanted off the settled sediment and filtered using a 20 ml glass syringe and disposable 0.45 µm filters (Millipore). The absorbance of each sample at 460 nm using a 1 cm cell was then measured using a

spectrophotometer (Pharmacia-LKB Novaspec II) calibrated against standard solutions prepared from 'humic acid'-sodium salts (Aldrich). However, since this is an operationally defined method, the results may not be absolutely accurate, but should enable reproducible comparison between sediment samples.

3.2.5 Sediment iron and manganese oxyhydroxides

Oxyhydroxide coatings on sediment particles are known to be important in the sorption of metals, and may be estimated by extraction into 1M HCl (Bryan et al., 1985; Luoma and Bryan, 1981). For this method, 2 ml aliquots of sediment slurry were pipetted into 20 ml glass vials; 10 ml of 1M HCl added, and stirred for 2 h on a magnetic stirrer using a PTFE coated 'flea'. Extracts were then decanted off the settled sediment and filtered using a 20 ml glass syringe and disposable 0.45 µm filters (Millipore). Fe and Mn concentrations were determined by flame atomic absorption spectrometry (Varian AA20) and the results presented as the concentration in dry sediment.

3.2.6 Salinity

Salinity of seawater samples was determined chemically using a digital chloridometer (Hakkebuchler Instruments Inc.). This utilises the combination of silver ions and chloride ions in a quantitative reaction that results in the precipitation of insoluble silver chloride. As the equivalence point of the reaction is reached the change in current flow is detected by the instrument circuitry and the titration reaction stops. Results are displayed as millequivalents of chloride per litre which may be calibrated against standard I.A.P.S.O. seawater (Institute of Oceanographic Sciences) and converted to salinity units using the Knudsen equation (Strickland and Parsons, 1972):

$$S\text{ ‰} = 0.030 + 1.8050\ Cl^{-}\text{ ‰}$$

3.2.7 pH

Seawater pH measurements were made using a benchtop laboratory pH meter (model 7020, Electronic Instruments Ltd.) calibrated against standard buffer solutions.

3.3 Factors Affecting TBT Partitioning

3.3.1 Time

Using the standard sediment-water partitioning protocol described, partitioning of ^{14}C-TBTCl between Avon estuary sediment and seawater was determined after periods of mixing from 10 minutes up to 24 hours. The results for sediment and water concentrations, and the resultant K_d values are summarised in Table 3.2 and Figures 3.1 & 3.2. These results show that a high proportion of the available TBT is rapidly scavenged by the sediment phase (approximately 60% after 10 minutes), in agreement with the findings of (Harris and Cleary, 1987). The changes of TBT concentrations in both phases are described by logarithmic functions of time (Figure 3.1). The concentrations in seawater decrease with time, while those in the suspended sediment increase, with the initial rapid uptake by sediments followed by a slower accumulation of TBT from the water. This may be explained by considering the mass transfer steps involved in establishing sorption equilibrium, and the different absorptive sites available in the sediment.

Table 3.2 Summary data for variation of K_d with time

Time (mins)	TBT in Seawater (ng l^{-1})	TBT in Sediment (ng g^{-1})	Proportion of TBT in Sediment (%)	Kd (l kg^{-1})
10	129.05	200.1	57.36	1551
10	118.65	190.8	60.88	1608
10	117.53	186.4	60.41	1586
30	126.80	206.8	62.92	1631
30	111.69	205.6	63.25	1841
30	109.69	205.4	64.25	1872
60	98.97	223.4	72.37	2258
60	106.58	222.1	68.99	2084
60	96.04	206.5	66.16	2150
180	78.49	254.2	77.78	3238
180	73.08	224.9	73.18	3078
180	79.26	224.9	72.01	2838
540	75.13	302.4	91.93	4025
540	67.56	261.0	78.28	3864
540	60.52	268.7	85.44	4439
1440	42.18	307.8	88.87	7298
1440	46.85	285.9	85.06	6102
1440	42.69	284.7	87.42	6670

Mass transfer can be taken to involve 3 main steps:

1. Diffusion of the solute to the sediment.

2. Sorption on to the surface of the sediment.

3. Movement to, and sorption on to the internal surfaces of sediment particles.

(Podoll and Mabey, 1987) considers that for a well mixed system and a low molecular weight (<500) hydrophobic solute, steps 1 and 2 are very fast, with a combined half life of the order of minutes. (Karickhoff, 1980) concluded that sorption onto the internal surfaces of sediment particle was slower, and probably limited by diffusion within the pore structure of the sediment organic fraction, and that the half-life of this process was of the order of hours in a well mixed system.

The results for TBT adsorption (Figures 3.1 and 3.2) would appear to be explained by this model. Furthermore, the rapid adsorption of TBT has a strong environmental significance in that it demonstrates that partitioning can occur within the period of time that sediments and water may be re-suspended under estuarine conditions at periods of maximum tidal flow. However, it would appear that in order to achieve equilibrium partitioning, longer periods of mixing would be required, which may not always be achieved under natural conditions.

Figure 3.1 Variations of mean TBT concentrations in water and sediments with time during partitioning.

(Error bars show ± 1 S.D.)

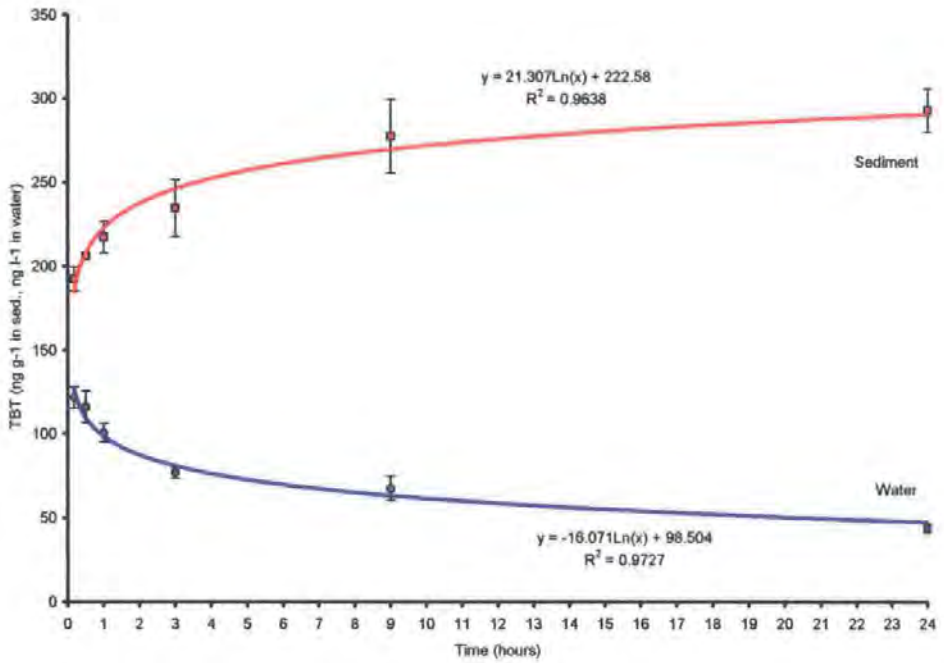
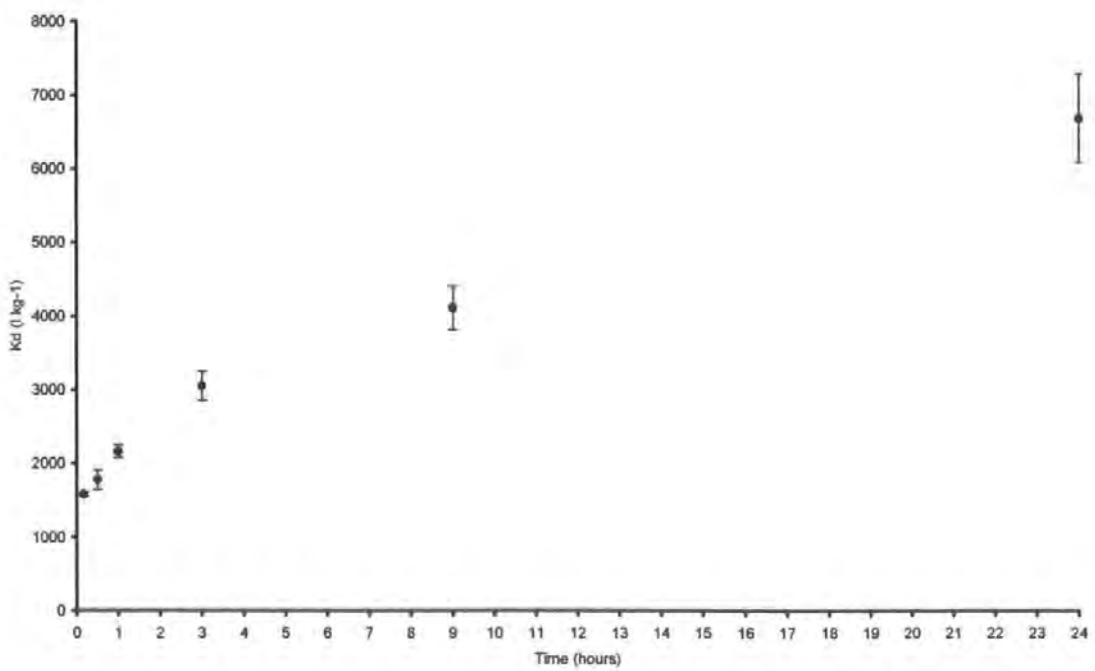


Figure 3.2 Variation of the equilibrium partition coefficient (K_d) with time.

(Error bars show ± 1 S.D.)



3.3.2 TBT Concentration

Many of the published partition coefficients (Table 3.1) were derived from experiments where the levels of TBT used were considerably in excess of those found in the environment, even in the most severely contaminated situations. It was therefore considered necessary to determine whether or not the K_d value changed as the level of TBT in the sediment/water system was varied.

Using the standard protocol described earlier, sediment/water mixtures were dosed at five different TBT concentrations (2 replicates at each level). After partitioning was complete, the extraction and analysis of sediments and water was performed as described (Chapter 2). The results are presented in Table 3.3. A plot of the equilibrium concentrations of TBT in seawater and sediments gives the adsorption isotherm (Figure 3.3). As mentioned earlier, sorption isotherms can take several forms, depending on the nature of the solute, its solubility and concentration, and the nature of the sorbent. Linear isotherms are common for low molecular weight organic solutes with low aqueous solubilities, at very low concentrations (< 50% of their aqueous solubility). The isotherms are linear because the solute/sediment interactions are non-specific; i.e. the solute does not interact strongly with specific surface sites on the sediment. However, the isotherm for TBT is non-linear, its slope decreasing as concentration increases, and is described by a Freundlich equation:

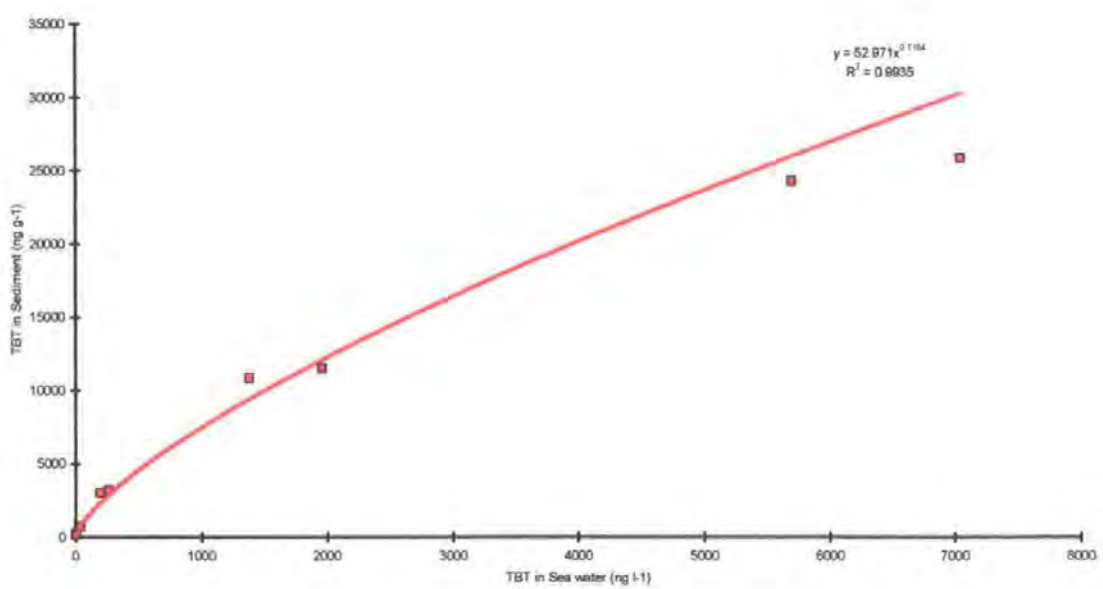
$$C_{sed} = 52.97 * C_{water}^{0.7164}$$

Thus the Freundlich parameters K_F and n for this sediment equate to 52.97 and 1.396 respectively. Such non-linear isotherms indicate that the solute is adsorbing to specific sites on the sediment, and are most common for ionizable organic solutes (such as TBTCl) with n>1, where solute/sediment interaction energies decrease as the higher energy adsorption sites gradually become filled as more solute is added (Podoll and Mabey, 1987).

Table 3.3 Summary data for variation of K_d with TBT concentration

TBT Dose (ng)	TBT in water (ng l^{-1})	TBT in sediment (ng g^{-1})	K_d (l kg^{-1})
75	6.40	189.7	29635.94
75	7.36	182.8	24831.52
300	28.46	705.2	24777.93
300	43.98	704.8	16025.24
1500	198.70	2987.6	15035.73
1500	268.80	3159.7	11754.84
6000	1385.40	10811.0	7803.52
6000	1961.00	11470.0	5849.06
15000	5704.00	24247.0	4250.88
15000	7050.00	25797.0	3659.15

Figure 3.3 Adsorption Isotherm for TBT on Estuarine Sediment



As a result, the value of the partition coefficient (K_d) is not constant at all TBT concentrations, but decreases as the TBT concentration in sea water increases. This is a feature of solutes with a Freundlich parameter $n > 1$. The partition coefficient (K_d) from this experiment varies significantly (P = 0.0047, ANOVA) in relation to the level of TBT used, indicating that K_d values which have been calculated from experiments using high TBT doses, and which result in unrealistic levels of TBT in both the sediment and water phases, may not be reliably used as predictors of the behaviour of TBT in the environment. The variation of K_d in relation to the concentration of TBT in seawater as experimentally determined here, follows a distinct logarithmic function (Figure 3.4).

Figure 3.4 Variation of K_d with TBT concentration in seawater

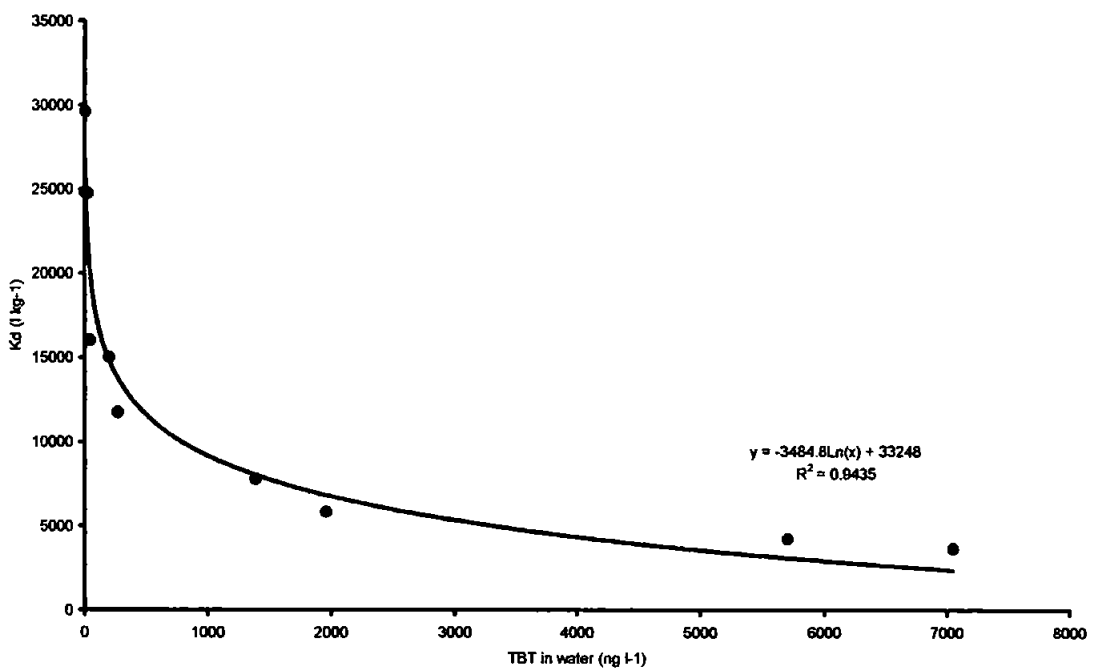
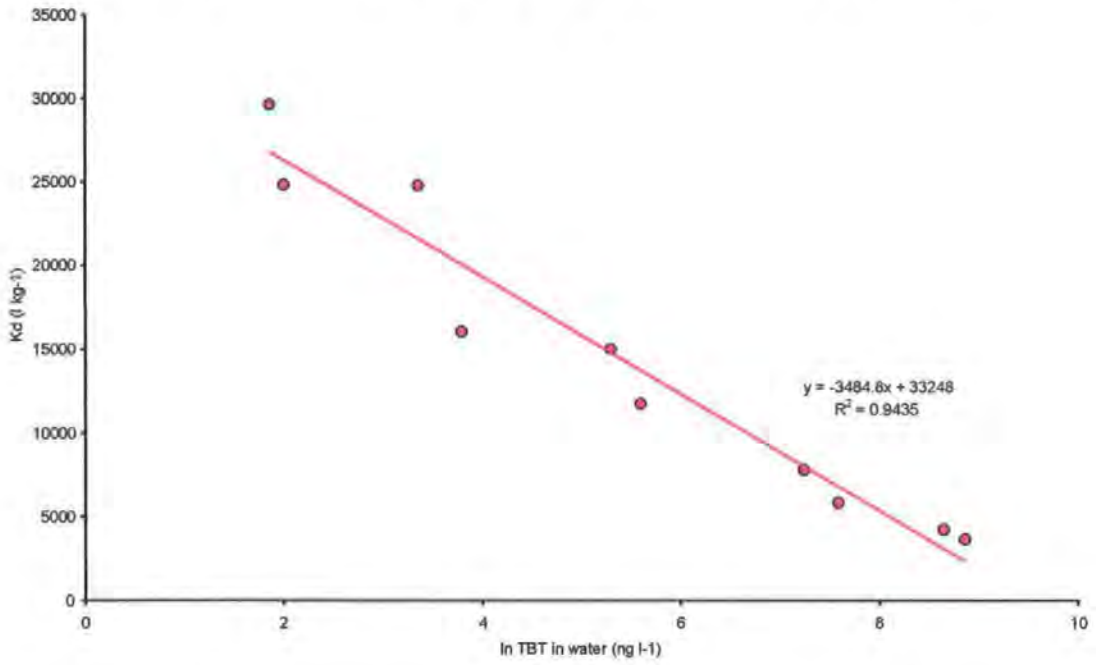


Figure 3.5 Variation of Kd with log^e TBT concentration in seawater



When the results are plotted with the concentration of TBT in the water on a log^e (ln) scale (Figure 3.5), it can be seen that a linear relationship exists (r^2= -0.9435) between the value of K_d and the concentration of TBT in the water, described by:

$$K_d = 33248 - \{3484.8 \text{ (ln TBT in sea water)}\}$$

The equation of this line may be different for other types of sediment, since it has been shown in other areas of this study that K_d varies from one sediment to another. However, it is apparent that K_d varies systematically in relation to the concentration of TBT in seawater, which, in experimental procedures, is dependent on the TBT dose used.

With these results in mind, further experiments designed to study the partitioning behaviour of TBT have been conducted using dose levels that result in environmentally realistic concentrations of TBT, while remaining well within the range of measurement of the techniques used.

3.3.3 Suspended Sediment Load

In the course of designing experiments to measure K_d values of different sediments, or identical sediments under differing environmental conditions, it was apparent that if sediments were to be used in a condition as similar as possible to that found in the field, they would have to be handled as a wet slurry. While this does not constitute a problem in terms of calculating the dry weight equivalent of the sediment, it does mean that there is scope for slight variation in the amount of sediment initially used when the experiment is set up, due to the difference in wet/dry ratios of different sediments. As a result, the effect of changing the amount of suspended sediment on the value of K_d was investigated. The results are also highly relevant for estuarine conditions, where suspended sediment loads may change significantly both spatially and temporally.

The standard experimental protocol was used, but with 5 levels of suspended solids from 0.045 to 1.3 g l^{-1}. These sediment loadings were chosen to be representative of the range encountered in a macro-tidal estuary such as the Tamar.

These results are presented in Table 3.4 and show significant differences in the concentrations of TBT in both the sea water ($P < 0.0001$, ANOVA), and the sediment ($P = 0.0002$, ANOVA).

Table 3.4 Summary data for variation of K_d with suspended sediment load

Sediment Load	TBT in Sea water		TBT in Sediment		K_d
(g l^{-1})	(ng L^{-1})	(%)	(ng g^{-1} dry weight)	(%)	(l kg^{-1})
0.0396	190.54	63.51%	2651.98	35.05%	13918
0.0503	177.62	59.21%	2162.45	36.27%	12174
0.1387	130.35	43.45%	1214.23	56.14%	9315
0.1345	116.88	38.96%	1280.56	57.42%	10957
0.2797	88.18	29.39%	813.21	75.82%	9222
0.2722	93.43	31.14%	785.22	71.25%	8405
0.5378	43.14	14.38%	510.29	91.48%	11829
0.5369	50.77	16.92%	492.89	88.20%	9708
1.3072	22.08	7.36%	210.10	91.55%	9516
1.3084	18.39	6.13%	212.92	92.86%	11581

The concentrations in each phase decline as the amount of suspended solids increase (Figures 3.6 and 3.7), and a progressively greater proportion of the total TBT available becomes adsorbed - from an average of 36.75% at 0.045 g l^{-1} suspended solids to 93.18% at

Figure 3.6 Variation of TBT in seawater with suspended sediment load

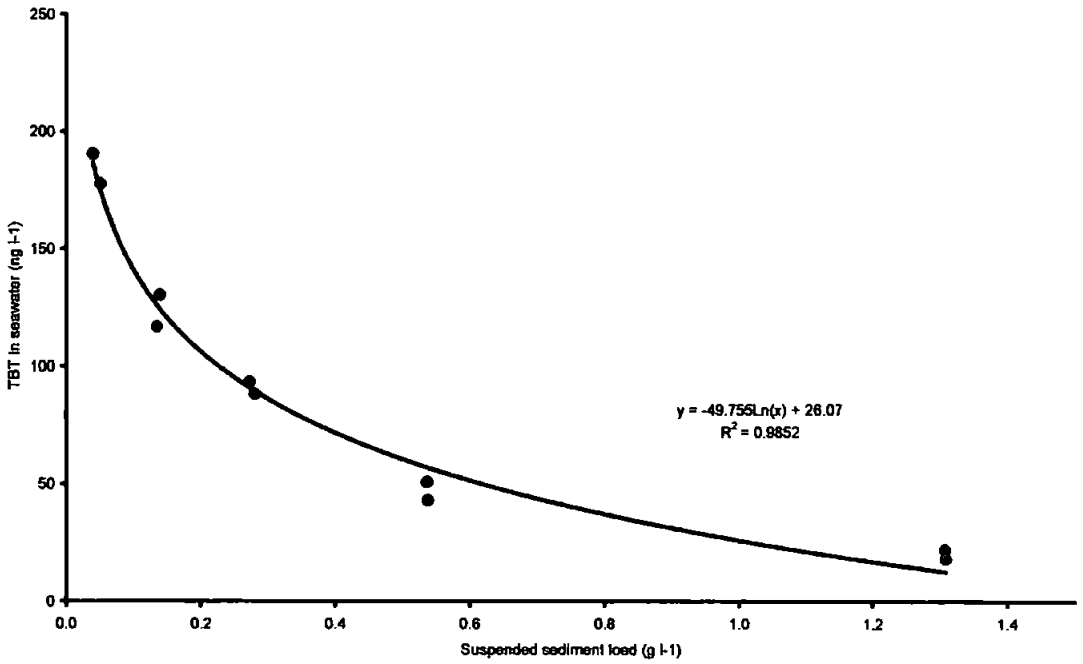


Figure 3.7 Variation of TBT concentration in suspended sediment with the suspended sediment load

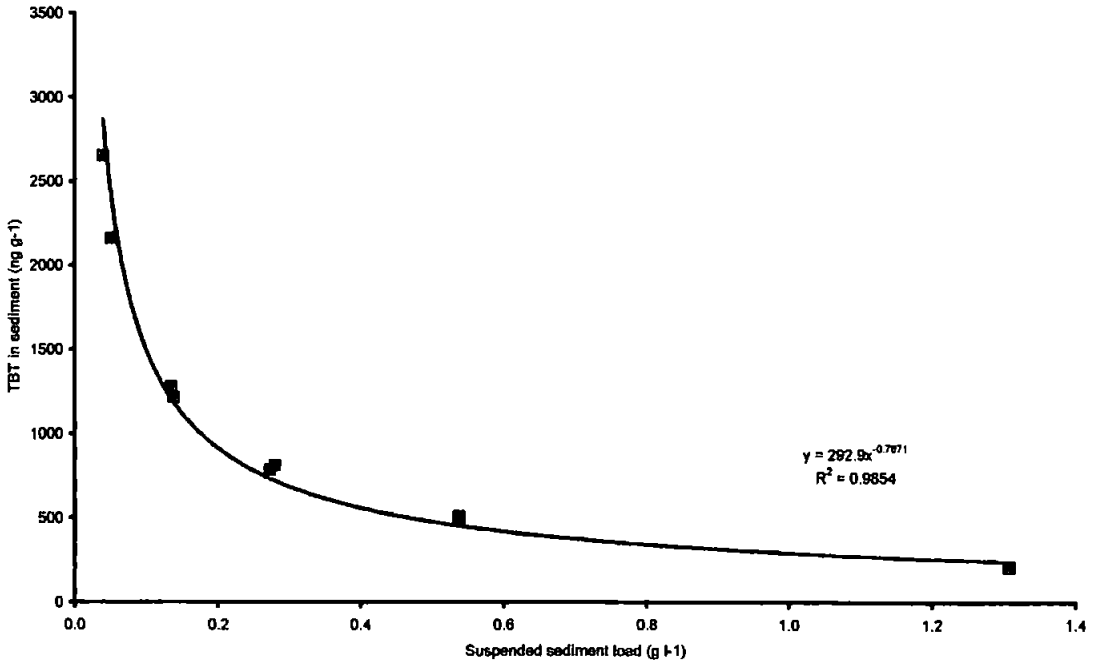


Figure 3.8 Proportion of total TBT in each phase with increasing suspended sediment load

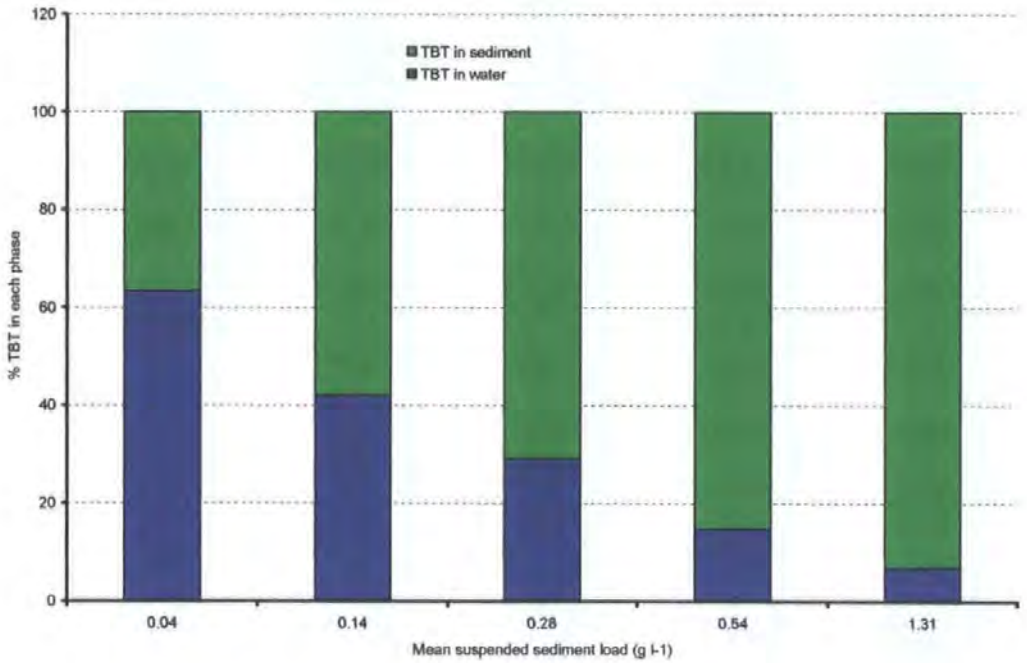
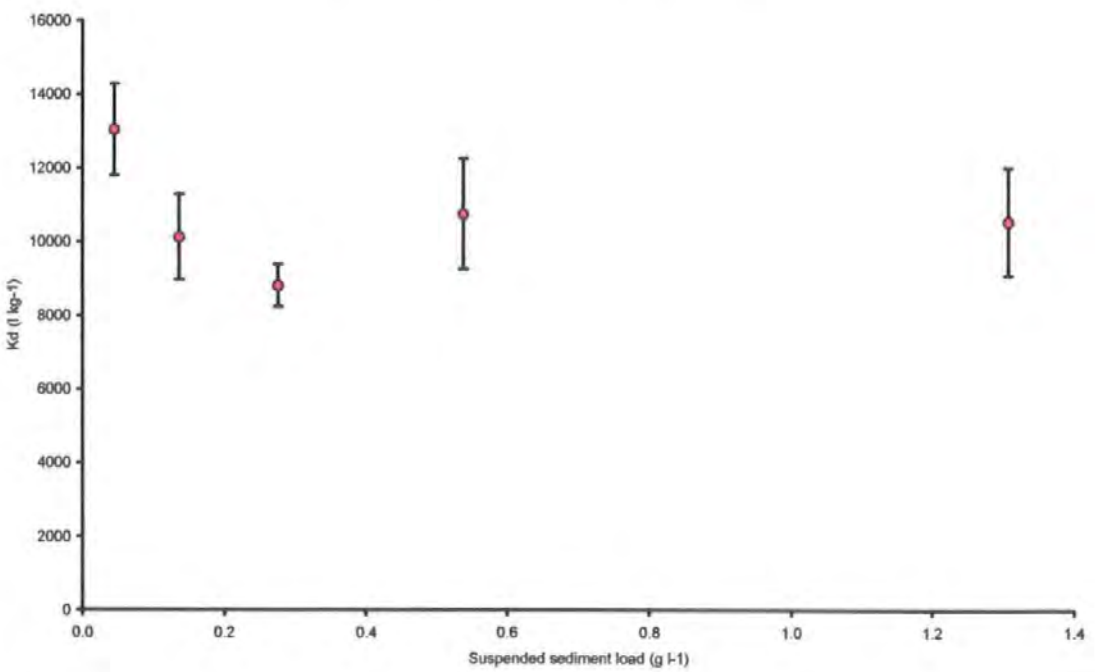


Figure 3.9 Variation of K_d with suspended sediment load.

(Error bars show mean ± 1 S.D.)



1.308 g l^{-1} (Figure 3.8) - although the concentration in the sediment phase declines as more sediment is added. However, when these concentrations are combined, the resultant K_d values show no significant difference (P = 0.1231, ANOVA) across the range of suspended sediment loads tested (Figure 3.9).

Considering that these results compare K_d values across a 30-fold increase in sediment load, and the measured change in K_d across the range is less than 2-fold (max. = 13918, min = 8405), it seems likely that in subsequent experiments, the variation in K_d due to differences in sediment loading (as a result of the different wet/dry ratios of different sediments), will be negligible.

However, there does appear to be a trend towards higher K_d values at very low levels of suspended sediment (Figure 3.9). Similar variation of K_d with suspended sediment concentration has been observed previously for several classes of compounds (heavy metals, hydrophobic organics and radioactive substances), and sorbent types including clays, sediments, soils and sewage sludges (O'Connor and Connolly, 1980). The diversity of solute/sorbent combinations that exhibit such behaviour tends to exclude explanations based on specific chemical interactions, and suggests a non-specific, perhaps physical explanation. (Voice and Weber, 1985) proposed an explanation of this effect attributed to a transfer of sorbing, or solute-binding, material from the solid phase to the liquid phase during the course of partitioning experiments. It was proposed that this material, whether dissolved, macromolecular, or micro particulate in nature, was not removed from the liquid phase during separation procedures, and was capable of stabilising the solute, in solution. The amount of this material in the liquid phase would be proportional to the amount of solid phase present. Therefore the capacity of the liquid phase to accommodate solute depends on the amount of solid material present. Further experiments (Voice and Weber, 1985) suggested that the effect was due to micro-particles contributed by the solids, and not removed by filtration or centrifugation.

If this solids effect occurs in the environment, there could be significant implications with respect to the distribution and fate of TBT in estuarine environments, based on the following argument. In most estuaries, TBT inputs over the past 10 - 15 years have resulted in its

concentration in the bed sediments. These sediments effectively represent a high concentration of suspended solids. When they become re-suspended in the overlying water, by whatever means (tidal currents, bioturbation, dredging) the effective concentration of these contaminated solids are reduced by several orders of magnitude. If there is indeed a resultant increase in the partition coefficient at very low suspended solid concentrations (<100mg l^{-1}), and, if TBT levels in the overlying water are higher than that determined by the new K_d (for this level of solids), more TBT may become sorbed to the solid phase, and ultimately be transported back to the bed sediment. Here the solids concentration is effectively increased once more, K_d decreases and TBT may desorb into the interstitial water, where its diffusive flow would be slower than in the overlying water, thus leading to greater retention time in the sediment/water system than if K_d was constant.

Clearly this hypothesis is simplistic and would of course be dependent upon the environmental concentrations of TBT in all phases, together with the type and concentration of the sediment in consolidated and suspended form. Nevertheless, it perhaps illustrates the importance of partitioning criteria in understanding natural systems, and the effect on the bioavailability of contaminants to estuarine organisms. Further investigations of TBT partitioning at very low levels of suspended solids would be needed to determine whether this micro-scale cycling of TBT sorption/desorption is possible.

3.3.4 Salinity

3.3.4.1 Adsorption

Published data regarding the influence of salinity on the sediment-water partitioning behaviour of TBT are limited, and conflicting. Unger et al. (1987) demonstrated that K_d values for a Chesapeake Bay sediment *decreased* linearly, by a factor of 2 when the salinity of artificial sea water was increased from 0 to 35‰. Further investigations with two freshwater sediments showed a similar trend, but with much higher K_d values at very low salinities (<5‰) (Unger et al., 1988). In contrast, Harris and Cleary, (1987); and Randall and Weber, (1986) demonstrated that the proportion of TBT sorbed to particulates *increased* over the same range of salinities. It is possible that some of these differences could be attributed to the experimental procedures used, and the different media. Unger et al. (1987; 1988) used natural sediments which had not been pre-treated in any way, together with artificial seawater. Harris and Cleary, (1987) used natural sediments which had been freeze-dried, ground and sieved, and filtered natural sea water (diluted with distilled water); while Randall and Weber, (1986) used an artificial substrate comprised of hydrous iron oxide, and artificial sea water. Clearly these observed differences in sorption behaviour with changing salinity emphasise the need for investigations to be conducted in conditions as similar as possible to those found in the environment.

This study used the standard sediment-water partitioning protocol described earlier, modified to incorporate a range of salinities (filtered sea water diluted with filtered Tamar River water), to determine the influence of salinity on two estuarine sediments (St. Johns Lake and Calstock, both from the Tamar estuary in SW Devon) which had already been studied as part of the investigation into different sediment types, described later. In addition, the effect of salinity on TBT desorption was studied using sediment from the Avon estuary (South Devon) which had been artificially spiked with ^{14}C-TBT as part of a separate experiment.

The final (equilibrium) concentrations of TBT in the sea water and sediment phases (3 or 4 replicates at each salinity) for the two adsorption experiments, together with the respective K_d values are presented in Tables 3.5 & 3.6.

Table 3.5 Data for variation of K_d with salinity – St. John's Lake sediment

Salinity (% seawater)	TBT in Seawater (ng l^{-1})	TBT in Sediment (ng g^{-1})	K_d (L kg^{-1})
0	9.87	545.7	55260
0	15.05	537.3	35703
0	7.12	584.3	82095
0	8.16	566.5	69396
25	31.62	499.0	15783
25	40.19	506.3	12596
25	39.25	487.6	12424
25	37.00	485.2	13113
50	33.23	458.2	13788
50	33.50	514.2	15347
50	33.42	460.4	13775
50	33.55	483.9	14423
75	33.03	480.5	14549
75	34.68	474.1	13671
75	33.91	487.1	14365
75	31.00	460.0	14840
100	27.58	513.5	18617
100	28.99	506.1	17456
100	28.37	495.2	17456
100	28.17	505.9	17960

Table 3.6 Data for variation of K_d with salinity – Calstock sediment

Salinity (% seawater)	TBT in Seawater (ng l^{-1})	TBT in Sediment (ng g^{-1})	K_d (L kg^{-1})
0	16.32	603.1	36943
0	22.52	569.2	25278
0	21.19	609.8	28779
10	28.56	551.3	19303
10	30.87	572.4	18541
10	28.37	532.3	18763
25	32.15	517.3	16089
25	33.22	556.4	16751
25	37.77	564.0	14933
50	31.92	611.3	19154
50	39.11	603.9	15440
50	40.29	587.1	14573
75	37.11	601.0	16197
75	34.52	601.4	17421
75	35.19	579.2	16459
100	31.01	607.2	19580
100	31.40	605.8	19295
100	27.34	587.9	21501

For each sediment, these data were tested for significant differences by ANOVA together with between treatments (salinity) comparisons using Tukey's test. Examination of data for the experiment with St Johns Lake sediment showed significant heteroscedasticity, and was therefore log transformed prior to analysis. The data for Calstock sediment showed no such inequalities, and ANOVA was performed on the raw data. Results of these tests are shown in Tables 3.7 and 3.8.

The variations of mean TBT concentrations in water with salinity are shown in Figure 3.10. In both cases the mean concentration of TBT in the aqueous phase at zero salinity were significantly lower than at higher salinities. However, there were no significant differences between the mean concentrations at higher salinities. When sediment and water levels were combined to produce partition coefficients, both sediments showed highly significant differences between mean K_d values in fresh water and those under more saline conditions (Figure 3.12). In contrast to the previously reported effects of salinity on TBT partitioning (Unger et al., 1987) the data presented here show no linear trend in response to salinity changes. However, observation of the trends for TBT concentrations in water across the experimental range (Figure 3.10) suggest that TBT concentrations in the aqueous phase will be highest at intermediate salinities.

The mean concentrations of TBT in the sediment phase showed the opposite trend for St. Johns Lake sediment (Figure 3.11), with significantly higher mean concentrations at zero salinity, but with no difference between means as salinity increased. For Calstock sediment, changes in the adsorbed concentrations were not so clear, with only the concentrations at 25% and 100% sea water being significantly different from each other (Figure 3.11).

Table 3.7 Single factor analysis of variance for the effect of salinity on TBT levels in water and sediments; and on the resultant partition coefficient, K_d, for St John's Lake sediment.

(All data log transformed).

	df Effect	MS Effect	df Error	MS Error	F	p-level
TBT in Seawater	4	0.232783	15	0.004552	51.141	1.45E-08
TBT in Sediment	4	0.003161	15	0.000209	15.128	3.81E-05
K_d	4	0.286958	15	0.005554	51.667	1.35E-08

Tukey multiple comparisons for St John's Lake sediment

TBT in Seawater

Salinity (% Seawater)	0	25	50	75	100
0					
25	0.000149				
50	0.000149	n.s.			
75	0.000149	n.s.	n.s.		
100	0.000150	n.s.	n.s.	n.s.	

TBT in Sediment

Salinity (% Seawater)	0	25	50	75	100
0					
25	0.001074				
50	0.000210	n.s.			
75	0.000183	n.s.	n.s.		
100	0.005552	n.s.	n.s.	n.s.	

Equilibrium Partition coefficient K_d

Salinity (% Seawater)	0	25	50	75	100
0					
25	0.000149				
50	0.000149	n.s.			
75	0.000149	n.s.	n.s.		
100	0.000150	n.s.	n.s.	n.s.	

Table 3.8 Single factor analysis of variance for the effect of salinity on TBT levels in water and sediments; and on the resultant partition coefficient, K_d, for Calstock sediment.

	df Effect	MS Effect	df Error	MS Error	F	p-level
TBT in Seawater	5	116.906	12	8.137752	14.365	0.000104
TBT in Sediment	5	1902.949	12	323.114	5.889	0.005635
K_d	5	88918752	12	7435422	11.958	0.000254

Tukey multiple comparisons for Calstock sediment

TBT in Seawater

Salinity (% Seawater)	0	10	25	50	75	100
0						
10	0.017784					
25	0.000642	n.s.				
50	0.000239	0.049598	n.s.			
75	0.000375	n.s.	n.s.	n.s.		
100	0.011152	n.s.	n.s.	n.s.	n.s.	

TBT in Sediment

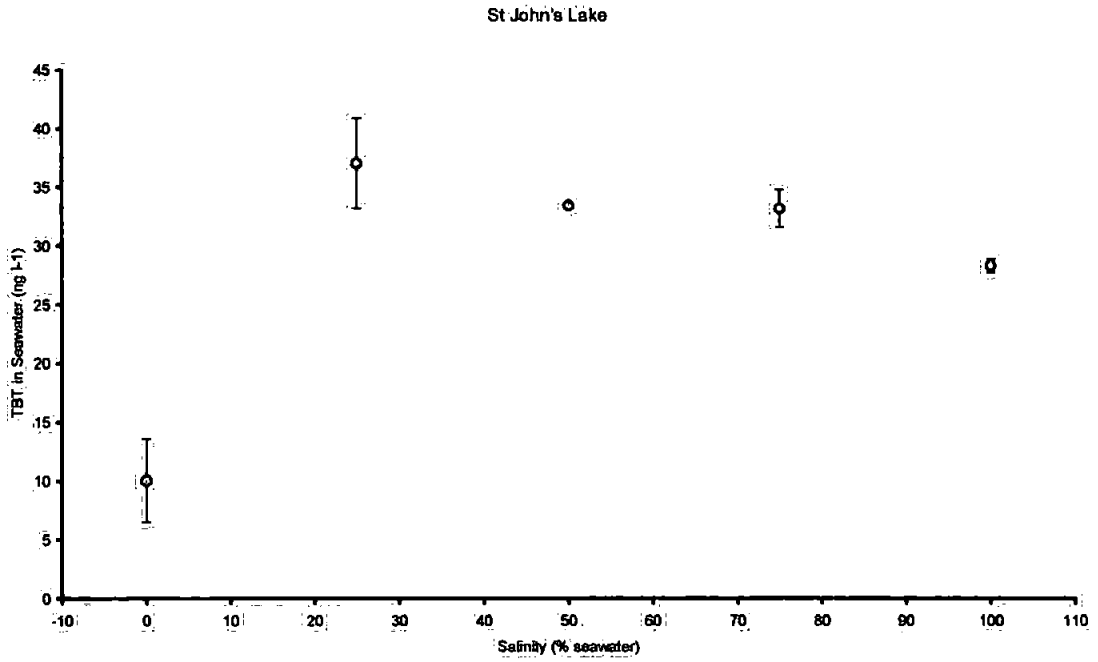
Salinity (% Seawater)	0	10	25	50	75	100
0						
10	n.s.					
25	n.s.	n.s.				
50	n.s.	n.s.	0.02649			
75	n.s.	n.s.	n.s.	n.s.		
100	n.s.	n.s.	0.027939	n.s.	n.s.	

Equilibrium Partition coefficient K_d

Salinity (% Seawater)	0	10	25	50	75	100
0						
10	0.002647					
25	0.000462	n.s.				
50	0.000576	n.s.	n.s.			
75	0.000679	n.s.	n.s.	n.s.		
100	0.006457	n.s.	n.s.	n.s.	n.s.	

Figure 3.10 Variation of mean TBT concentrations in water with salinity.

(Error bars show ± 1 S. D.)



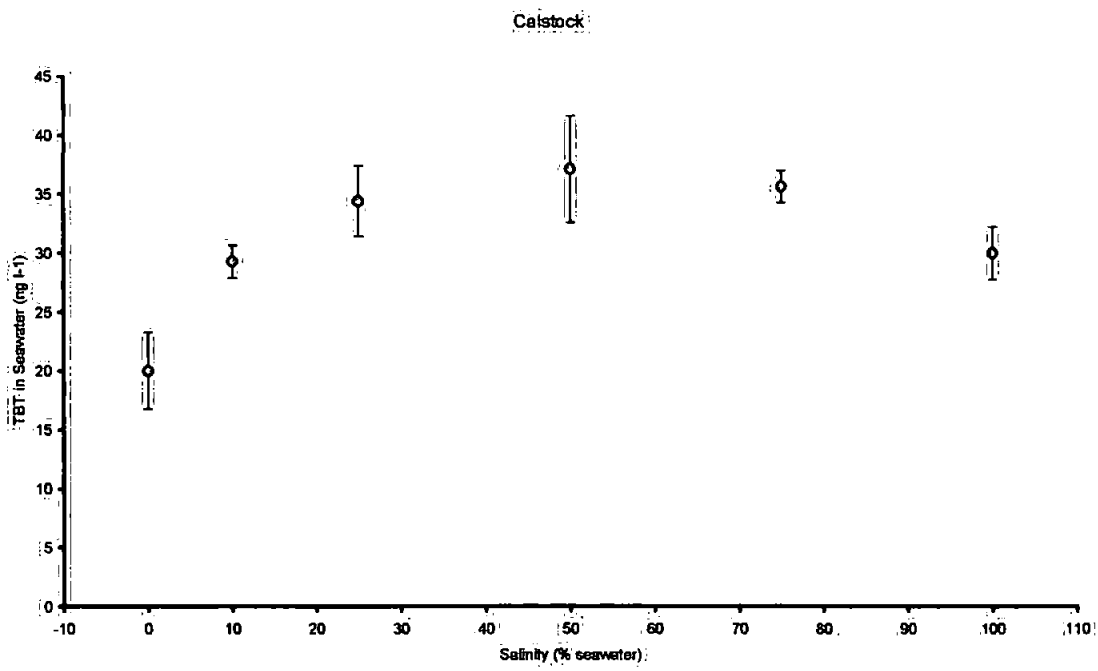


Figure 3.11 Variation of mean TBT concentrations in sediment with salinity for St. Johns Lake and Calstock sediments

(Error bars show ± 1 S. D.)

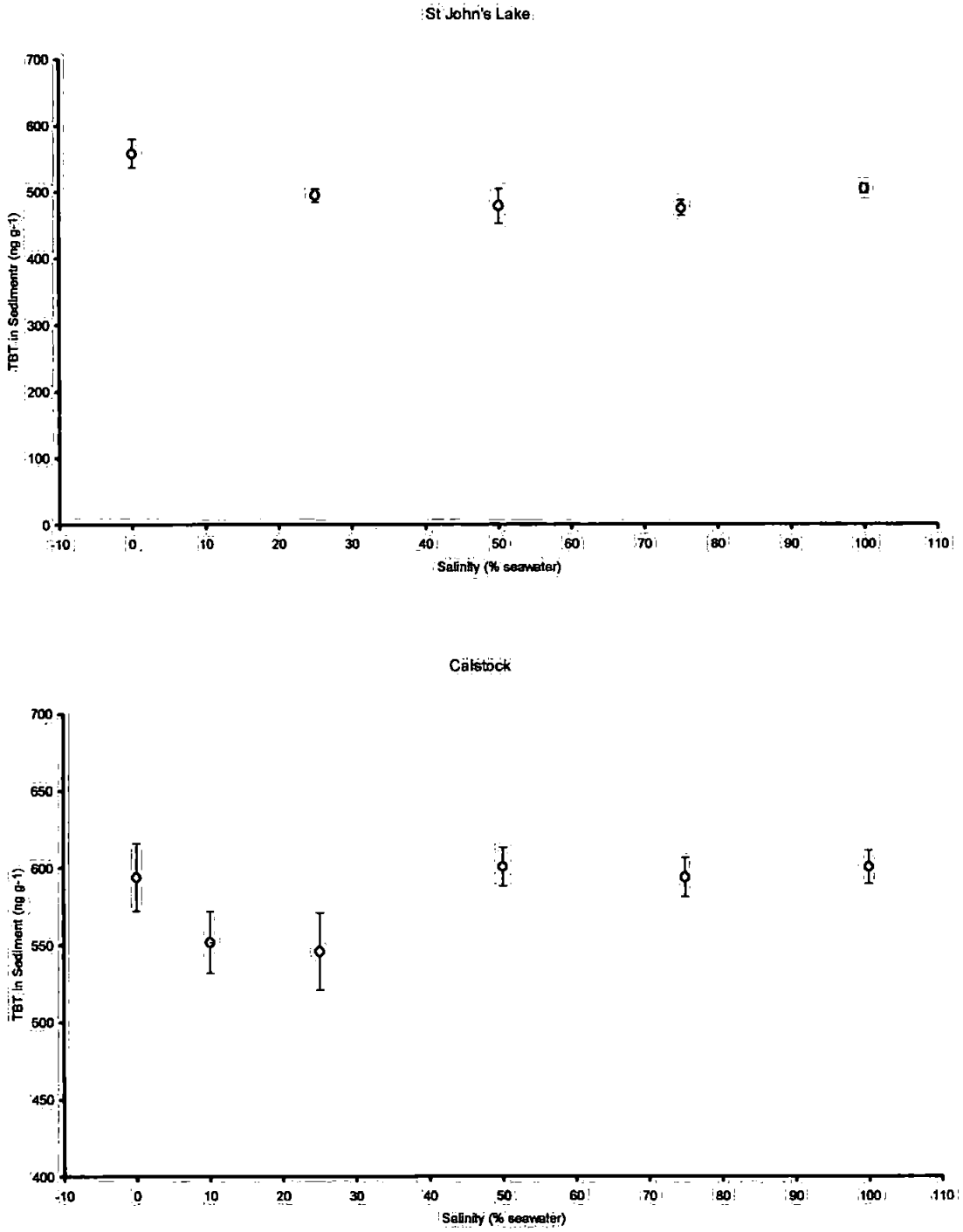
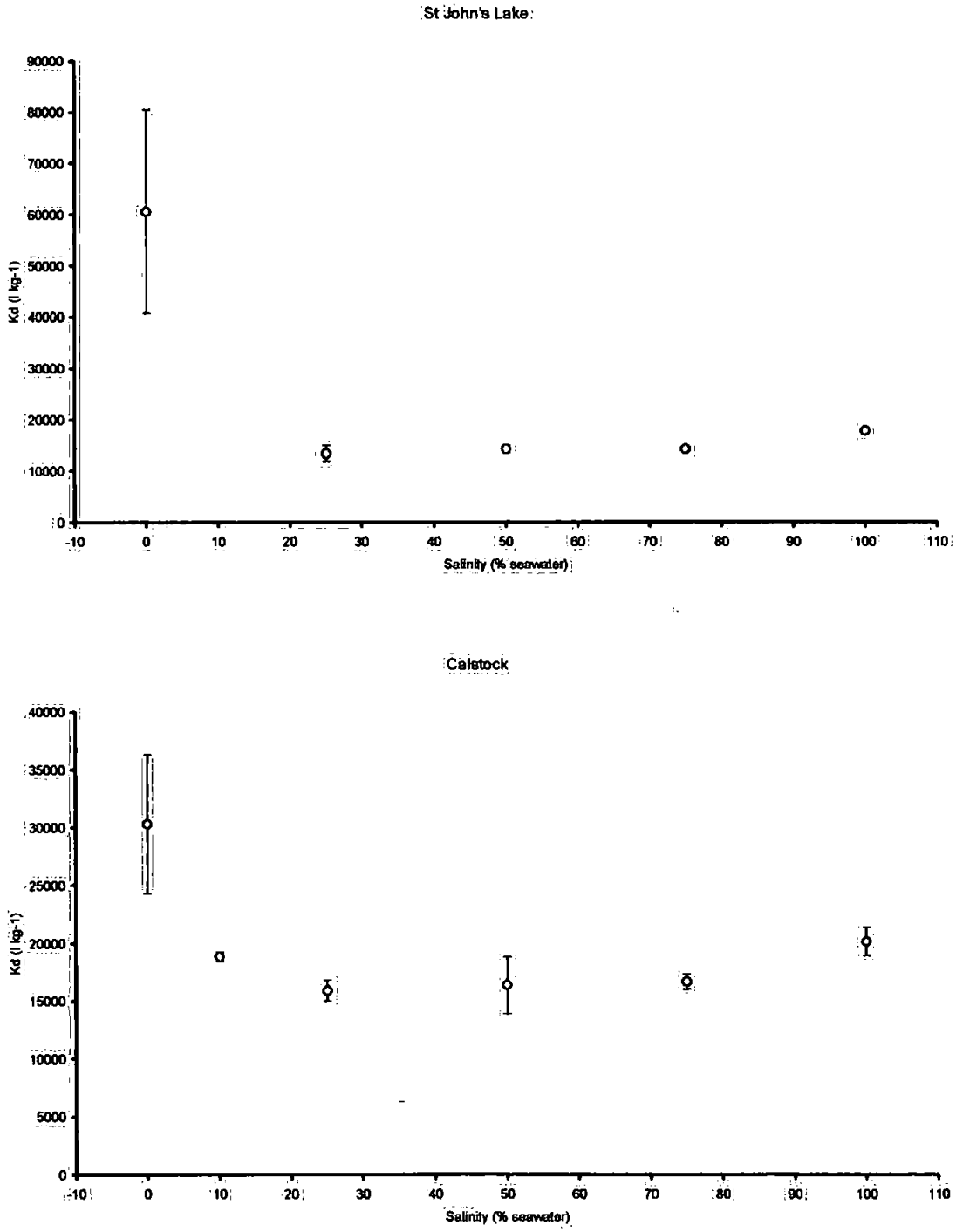


Figure 3.12 Variation of K_d with salinity for St. John's Lake and Calstock sediments.
(Error bars show ± 1 S. D.)



These results show a similar salinity response to those of Laughlin et al. (1986b) where the effect of salinity on the octanol-water partition coefficient (K_{ow}) and speciation of TBT in seawater were investigated. K_{ow} values between 5000 and 7000 were measured, with the lowest values occurring at 25‰ in a salinity range of 0 - 50‰. It was suggested that neither ionic strength nor pH changes associated with the dilution of seawater exerted the predominant effect. Instead it was proposed that at low salinity, the increasing stabilisation of charged species (TBT^+) in solution with increasing [Cl^-] was significant, resulting in a decreasing K_{ow}. At salinities above 25‰ increasing [Cl^-] shifts the TBT ionic speciation towards TBTCl with lower solubility of this undissociated species and a concomitant increase in K_{ow}.

Although the results of this investigation provide no indication of TBT anionic speciation under near natural conditions, the similarity of these results with those of Laughlin et al. (1986b) suggest that this rationale could be a plausible explanation of the effect of salinity on TBT partitioning between sediments and estuarine waters.

3.3.4.2 Desorption

If TBT sorption is reversible i.e. adsorption and desorption follow the same Freundlich isotherm, then desorption of TBT from sediments should also be greatest at intermediate salinities. To test this hypothesis, sediments from the Avon estuary (South Devon) which had been artificially spiked with ^{14}C-TBT (approximately 0.75 μg g^{-1} dry weight) as part of a separate investigation, were re-suspended under conditions similar to those used in the adsorption experiments, with the exception that sea water dilutions were made using tap water instead of river water (distilled water was considered to be too acidic for low salinity dilutions).

The resulting equilibrium concentrations of TBT in the aqueous and sediment phases and the respective K_d values are presented in Table 3.9. An analysis of variance with multiple comparisons between groups shows some significant differences with increasing salinity for these data (Table 3.10). These effects of salinity on TBT desorption are shown in Figures 3.13-3.15.

Table 3.9 Data for variation of TBT desorption with salinity – Avon sediment

Salinity (% Seawater)	TBT in Seawater (ng/L)	TBT in Sediment (ng/g)	Kd (L/kg)
0	18.05	792.1	43879
0	20.96	786.0	37505
0	17.19	792.0	46082
0	18.22	807.0	44301
5	23.61	788.4	33394
5	22.19	812.5	36622
5	23.37	790.8	33832
5	23.70	778.7	32858
10	25.79	774.1	30013
10	22.52	792.2	35182
10	21.94	790.0	36007
10	22.33	778.8	34875
25	23.68	754.0	31847
25	26.20	763.2	29125
25	22.75	767.3	33730
25	22.17	755.9	34093
50	18.11	766.2	42317
50	18.90	765.6	40514
50	23.70	793.3	33475
50	21.04	770.5	36620
100	16.00	786.3	49150
100	15.67	773.3	49335
100	16.57	766.2	46225
100	16.21	755.2	46586

Table 3.10 Single factor analysis of variance for the effect of salinity on desorbed TBT concentrations in water and sediments; and on the resultant partition coefficient, K_d, for Avon sediment.

	df Effect	MS Effect	df Error	MS Error	F	p-level
TBT in Seawater	5	37.31	18	2.65	14.07	0.000011
TBT in Sediment	5	718.97	18	123.42	5.83	0.002271
K_d	5	147824544	18	7968764	18.55	0.000002

Tukey multiple comparisons

TBT in Seawater

Salinity (% Seawater)	0	5	10	25	50	100
0						
5	0.0092					
10	0.0105	n.s.				
25	0.0038	n.s.	n.s.			
50	n.s.	n.s.	n.s.	n.s.		
100	n.s.	0.0002	0.0002	0.0002	0.0156	

TBT in Sediment

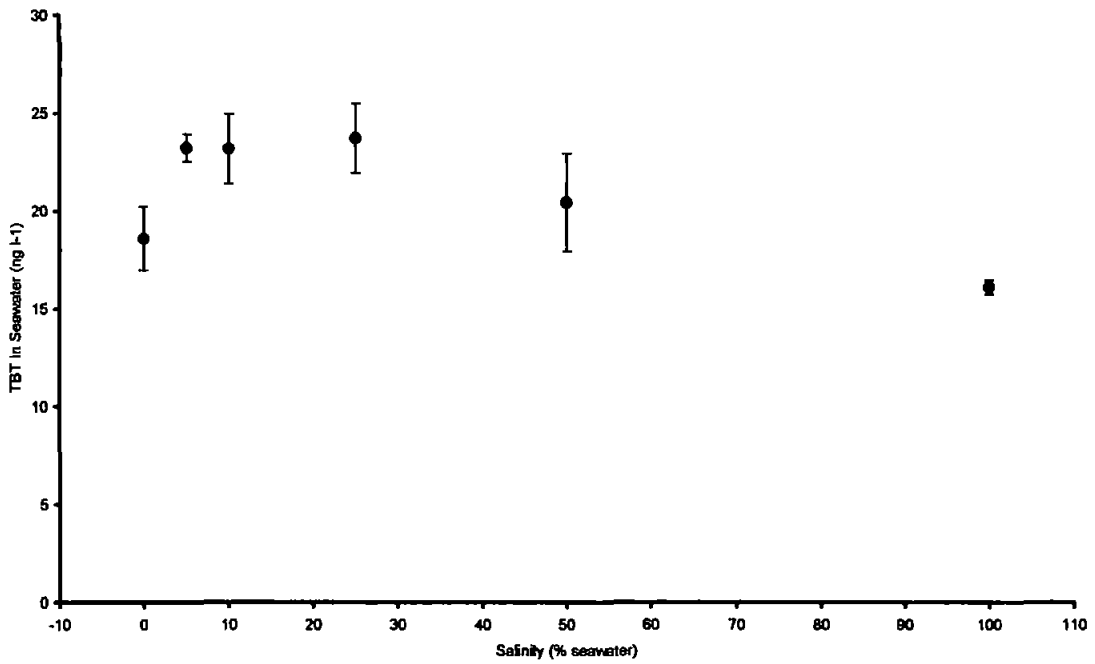
Salinity (% Seawater)	0	5	10	25	50	100
0						
5	n.s.					
10	n.s.	n.s.				
25	0.0045	0.0071	n.s.			
50	n.s.	n.s.	n.s.	n.s.		
100	n.s.	n.s.	n.s.	n.s.	n.s.	

Equilibrium Partition coefficient K_d

Salinity (% Seawater)	0	5	10	25	50	100
0						
5	0.0041					
10	0.0035	n.s.				
25	0.0006	n.s.	n.s.			
50	n.s.	n.s.	n.s.	n.s.		
100	n.s.	0.0002	0.0002	0.0002	0.0018	

Figure 3.13 Effect of salinity on the desorption of TBT from Avon estuary sediments to seawater

(Error bars show ± 1 S. D.)



The overall response is very similar to that found for adsorption, namely that the greatest concentrations of TBT are desorbed in the salinity range 5 - 25% seawater demonstrating TBT sorption to be reversible to some extent. Both the aqueous TBT concentrations (Figure 3.13), and the K_d values (Figure 3.15) at 5, 10 and 25% sea water show significant differences from those at 0 and 100% sea water, while the values at 50% are intermediate to the two extremes. The increase in aqueous TBT concentrations at low salinity is reflected by a concomitant fall in sediment levels (Figure 3.14), although in this case only the concentration at 25% sea water is significantly different from those at 0 and 5% sea water. The lower incidence of significant differences in sediment TBT levels may be attributed to the fact that the majority of the TBT in the system (94.17% to 96.17%) remains bound to the sediment phase across the salinity range, such that the relative changes are very small.

Figure 3.14 Effect of salinity on desorption of TBT from Avon estuary sediments

(Error bars show ± 1 S.D.)

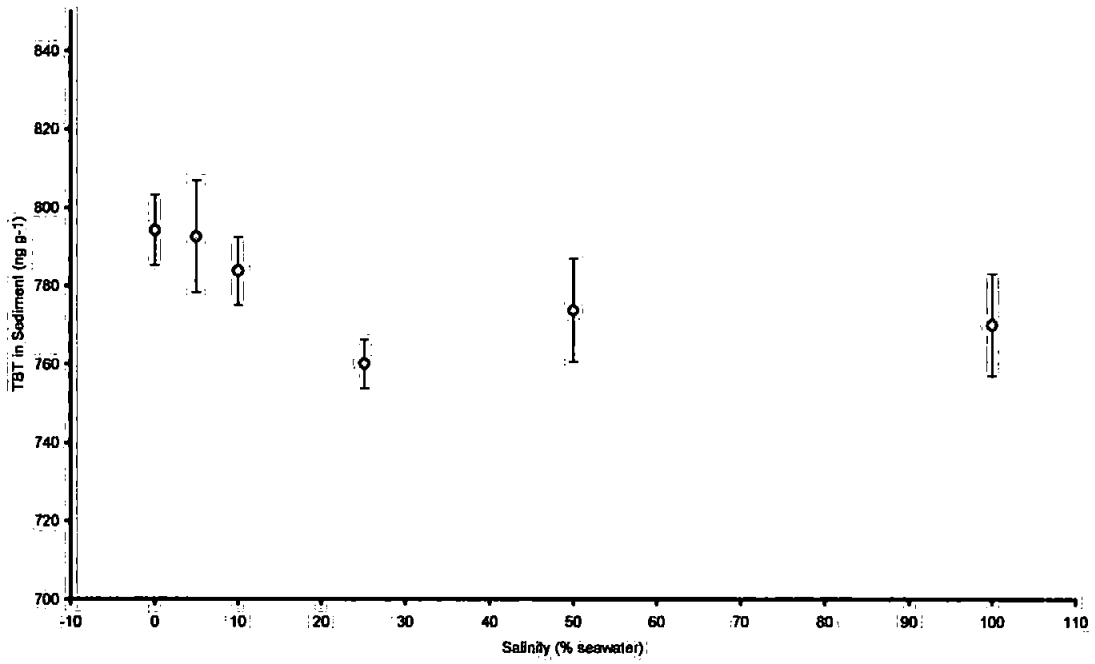
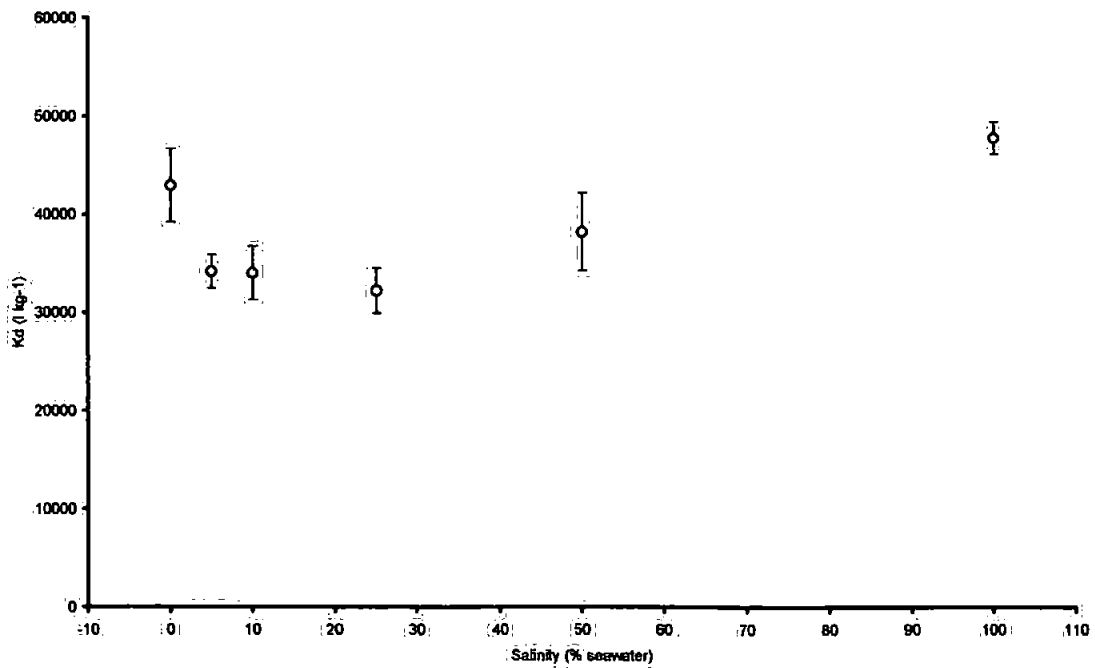


Figure 3.15 Effect of salinity on K_d for Avon estuary sediment during desorption

(Error bars show ± 1 S.D.)



3.3.5 pH

Although seawater is usually well buffered at around pH 8, less saline waters near the riverine end of estuaries may have a lower pH. In addition, greater pH ranges may be encountered locally as the result of acid or alkaline wastes (e.g. mine drainage or decaying vegetation).

This study investigated the effect of pH on the desorption of TBT from an estuarine sediment which had previously been spiked with 0.9 μg g^{-1} ^{14}C-TBT. Using identical methods to the TBT desorption versus salinity experiment, sediment aliquots were suspended in sea water for 24 hours at six pH levels between 4.65 and 9.18 (produced by the addition of either 0.1M HCl or 0.1M NaOH to 50% filtered sea water). Mean pH values are reported, based on readings taken at the beginning and end of equilibration, although there was little change during the course of the experiment.

Table 3.11 Data for variation of TBT desorption with pH

pH	TBT in Seawater (ng l^{-1})	TBT in Sediment (ng g^{-1})	K_d (l kg^{-1})
4.65	39.69	784.4	19762
4.68	40.09	761.9	19005
4.70	40.70	767.8	18863
4.70	40.42	781.9	19341
5.50	17.76	845.4	47599
5.50	16.03	799.4	49875
5.55	14.07	854.3	60723
5.60	18.22	860.7	47241
6.45	10.26	860.6	83883
6.00	9.46	867.6	91698
6.45	11.52	860.3	74665
6.55	8.57	864.8	100952
7.10	7.53	854.9	113545
7.15	8.71	853.6	97999
7.15	8.81	845.8	95971
7.15	6.85	841.0	122841
7.90	18.07	807.0	44664
7.90	16.77	802.1	47826
7.90	16.79	851.2	50712
7.90	18.31	846.0	46191
9.20	42.75	775.9	18152
9.20	45.20	748.4	16559
9.18	43.24	779.7	18032
9.18	42.74	766.1	17925

Table 3.12 Single factor analysis of variance for the effect of pH on desorbed TBT concentrations in water and sediments; and on the resultant partition coefficient, K_d.

(All data log transformed).

	df Effect	MS Effect	df Error	MS Error	F	p-level
Log TBT in Seawater	5	0.371093	18	0.001488	249.3492	5.8E-16
Log TBT in Sediment	5	0.001839	18	8.46E-05	21.72851	4.76E-07
Log K_d	5	0.423097	18	0.001561	271.1241	2.76E-16

Tukey multiple comparisons

Log TBT in Seawater

Mean pH	4.68	5.54	6.36	7.14	7.90	9.19
4.68						
5.54	0.0002					
6.36	0.0002	0.0002				
7.14	0.0002	0.0002	0.0252			
7.90	0.0002	n.s.	0.0002	0.0002		
9.19	n.s.	0.0002	0.0002	0.0002	0.0002	

Log TBT in Sediment

Mean pH	4.68	5.54	6.36	7.14	7.90	9.19
4.68						
5.54	0.0006					
6.36	0.0002	n.s.				
7.14	0.0002	n.s.	n.s.			
7.90	0.0043	n.s.	n.s.	n.s.		
9.19	n.s.	0.0003	0.0002	0.0002	0.0014	

Log Kd

Mean pH	4.68	5.54	6.36	7.14	7.90	9.19
4.68						
5.54	0.0002					
6.36	0.0002	0.0002				
7.14	0.0002	0.0002	n.s.			
7.90	0.0002	n.s.	0.0002	0.0002		
9.19	n.s.	0.0002	0.0002	0.0002	0.0002	

Figure 3.16 Variation of mean TBT concentrations in seawater with pH

(Error bars show ± 1 S. D.)

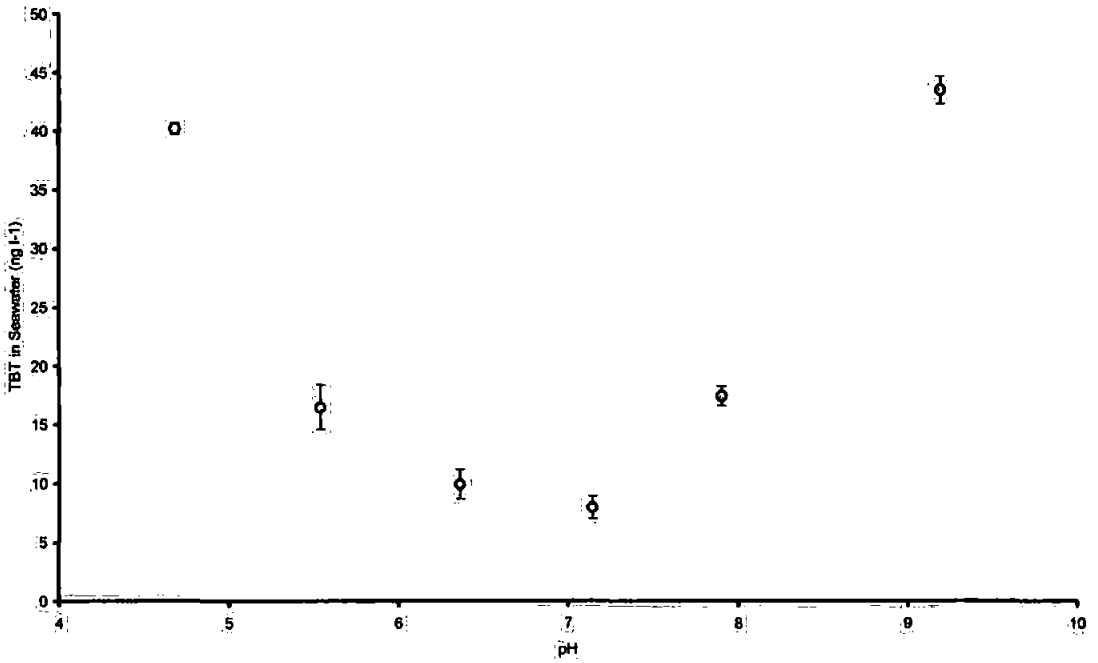


Figure 3.17 Variation of mean TBT concentrations in sediment with pH

(Error bars show ± 1 S. D.)

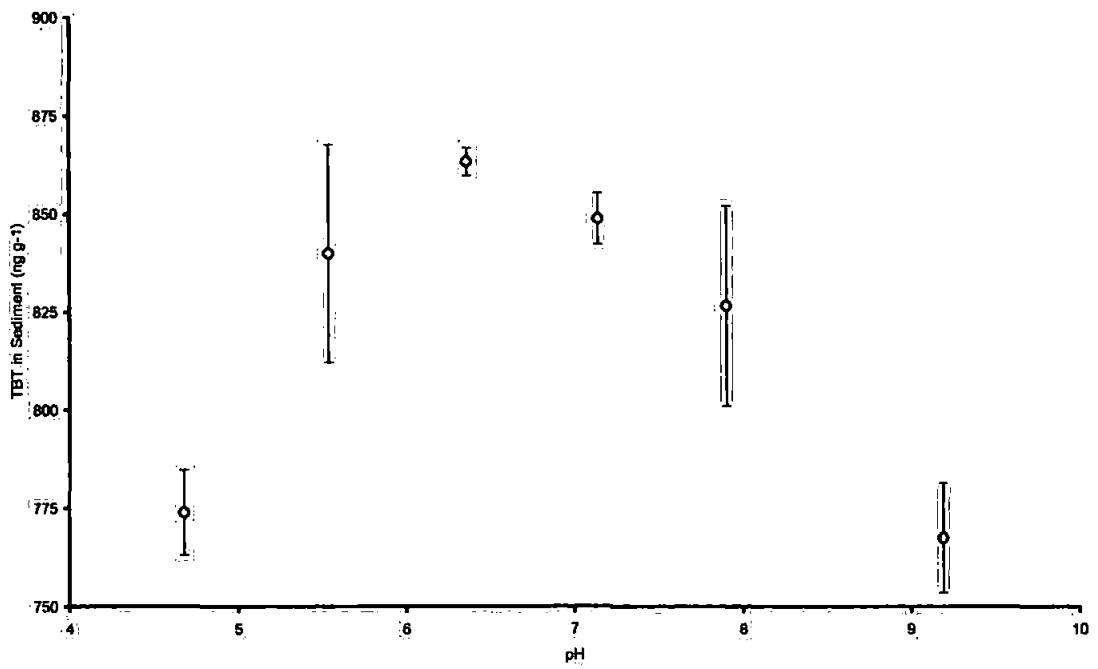
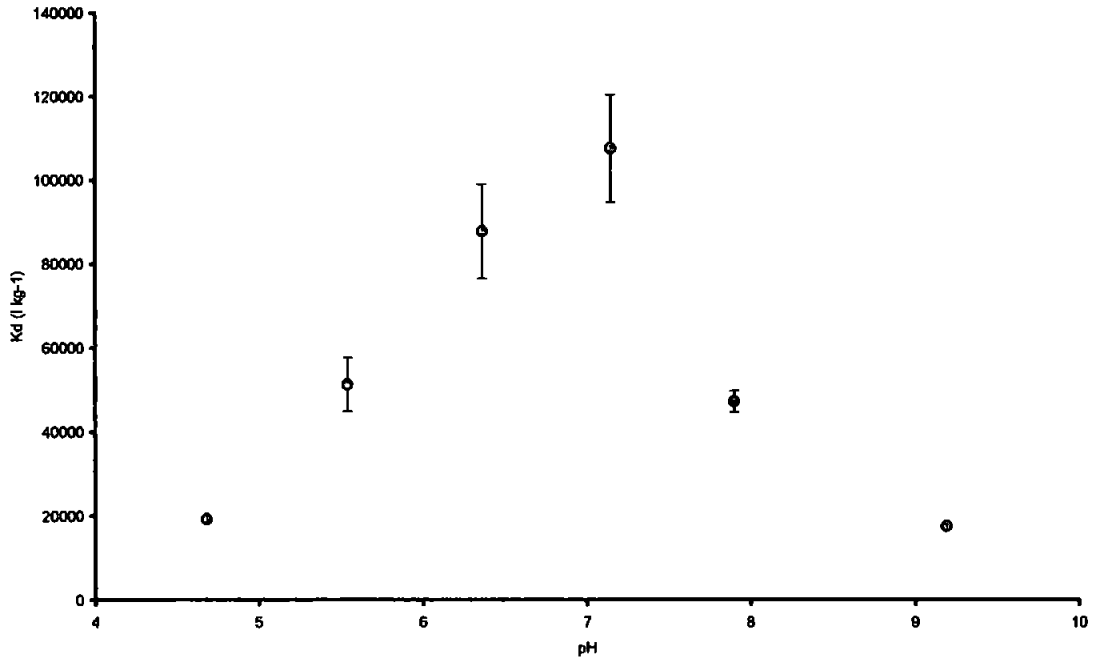


Figure 3.18 Variation of mean TBT partition coefficient (K_d) with pH

(Error bars show ± 1 S. D.)



The results at equilibrium are presented in Table 3.11 and a summary for analysis of variance of log-transformed data is shown in Table 3.12. Although the major proportion (88.5 - 97.7%) of TBT present was associated with the sediment phase across all pH values used, the amount of TBT desorbed to the aqueous phase was highly pH dependent ($P<0.0001$, ANOVA) with greatest release at each end of the pH range used (Figure 3.16).

The sediment associated TBT concentrations (Figure 3.17), and the respective K_d values (Figure 3.18) not surprisingly, show the reverse with maximum retention on the solid phase at a neutral pH of about 6 - 7. Both trends were highly significant ($P<0.0001$, ANOVA).

The reasons for this pH dependence are not certain, and may be due to a combination of factors. One explanation is that it reflects the solubility of TBT, which in distilled water is lowest at pH 6.0 - 6.6 (0.75 mg l^{-1}) and increases at both higher and lower pH (Maguire et al., 1983). Lower solubility in the mid pH range would then favour retention by particulates. The pH of the aqueous medium will also affect the nature of the solid phase adsorbent, although the nature and extent of pH induced modifications, to what has already been described as a complex, and non-homogeneous substrate, is undefined (Jenne and Zachara, 1987). The usual adsorption model for metal ions, where the sorption potential of a sediment for metals is primarily

determined by the quantity of amorphic oxides of Fe and Mn, and reactive particulate organic carbon (Salomons and Forstner, 1984) cannot entirely explain the results for TBT, since the butyltin compound is thought to be predominantly neutral, although it has been suggested that TBT occurs as the cation in sea water (Rzaev, 1979). The most likely situation is that TBT exists as a number of 'species' in combination with the major ionic components of sea water i.e. TBTCl, TBT_2CO_3, TBTOH, TBT 'aquo complex', etc. (Laughlin et al., 1986b). Thus its major character is that of a hydrophobic compound, which may also display ionic behaviour as a modification. For surface co-ordination reactions of metallic cations on hydroxylated mineral surfaces, pH is the master variable, and may affect the solute and the adsorbent. The pH (and salinity) may affect the anionic speciation equilibrium of TBT in solution via hydrolysis or protonation, thus affecting its solubility and, in addition will influence the surface charge properties of the sediment components. Surface charge is governed by the acidity of surface hydroxyl groups (SOH), and the zero point of charge (ZPC) is the pH where positive and negative charges balance, and is in turn determined by the intrinsic surface acidity constants of the sediment components, which may be further modified by organic coatings on the mineral surface. A pH change away from neutral may therefore increase the solubility of TBT, and in doing so, decrease its tendency to adsorb on to sediments. At the same time the surface charge of the sediment particles changes such that adsorption of ionic compounds or elements is favoured, as the nature of the adsorbing surface changes in response to pH and the ZPC of the sediment. In summary it would seem likely that TBT sorption is mediated by more than one pH dependent factor.

3.3.6 Sediment Type

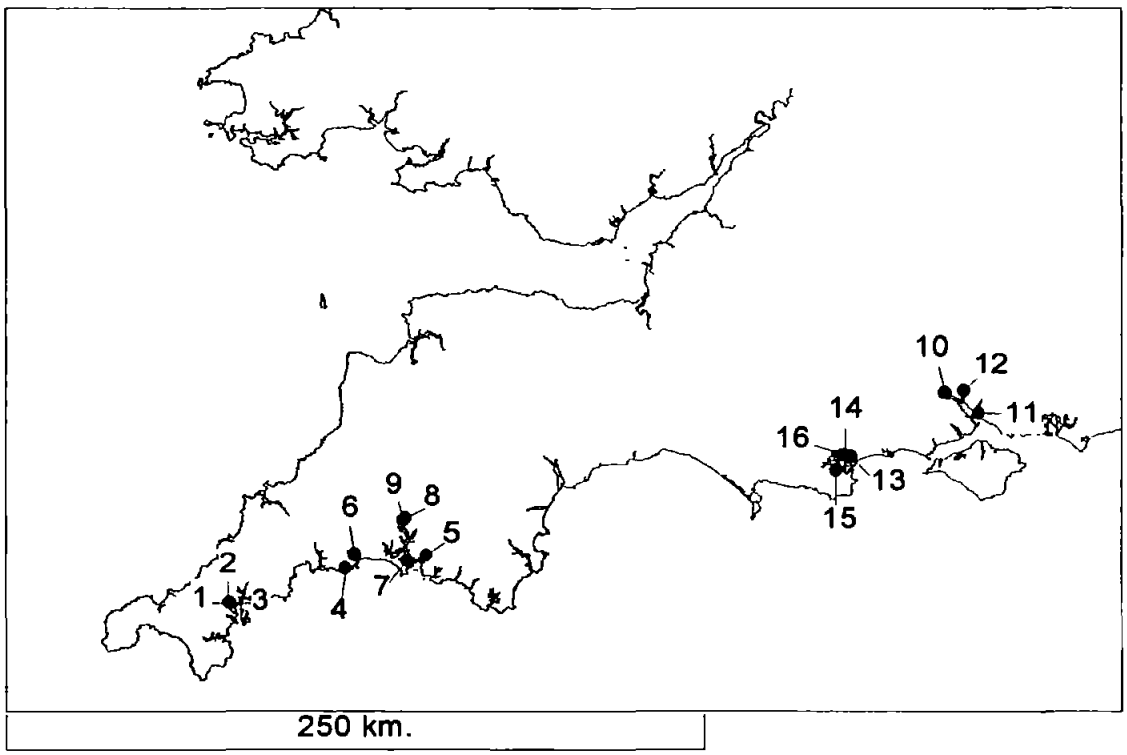
Given the variation in reported values of the sediment-water partition coefficient for TBT (Table 3.1), and the inherent heterogeneity of marine and estuarine sediments for reasons already discussed, the partitioning behaviour of TBT has been investigated in 16 different natural sediments collected from sites in the SW of the UK (Figure 3.19). The standard partitioning protocol was used to determine the value of K_d, with 5 replicates for each sediment type, and in addition, each sediment was characterised with respect to: grain size distribution; organic content; 'humic acid' content; and iron and manganese oxyhydroxide phases in order to deduce the significance of these parameters to TBT sorption. The values for these variables together with the mean K_d values for each sediment are presented in Table 3.13. The resulting values for the partition coefficient ranged over two orders of magnitude from 248 l kg^{-1} for Talland Bay (a sandy coastal sediment) to 24677 l kg^{-1} at Sterte, in Poole Harbour (a fine grained, organic rich estuarine mud).

Analyses of partitioning behaviour have been made by using correlation analysis between the results for partitioning studies and the sediment characteristics. The correlation matrix is shown in Table 3.14.

As may be expected, the grain size distribution of the sediments has a considerable effect on the magnitude of the partition coefficient. Significant negative correlations exist between K_d and the sand fraction in sediments, but significant positive correlations are shown for the fine and very fine silt fractions. There is no significant relationship between K_d and the clay fractions, suggesting that grain size alone does not explain the degree of adsorption, and may reflect the different adsorptive sites and mechanisms associated with different types of sediment particles.

Many neutral hydrophobic organic compounds have been shown to primarily adsorb to sediments with a high organic content (Karickhoff et al., 1979). A significant correlation exists here for the TBT partition coefficient and the organic content of the sediments (r^2=0.574, p=0.02), as may be expected, given its octanol-water partition coefficient – 5500 (Laughlin et al., 1986b). It can also be seen from Table 3.14 that the organic content itself is positively correlated with the silt fraction, and negatively correlated with the sand fraction. (Podoll and Mabey, 1987) noted that for partitioning studies in general, the sand fraction usually has a

Figure 3.19 Location of sites in southern England where sediments were collected for comparison of K_d with sediment parameters.



Site Number	Site	O.S. Map Reference	Estuary
1	Opposite Old Mine	SW803386	Fal estuary
2	Devoran	SW794388	"
3	Old Mine	SW803387	"
4	Talland Bay	SX250510	Coastal Site
5	Point Cottage	SX508554	Plym estuary
6	East Looe	SX250510	Looe estuary
7	St John's Lake	SX441535	Tamar estuary
8	Calstock	SX435686	"
9	Cotehele	SX424678	"
10	Totton	SU368133	Southampton
11	Warsash	SU489059	"
12	St. Denys	SU437139	"
13	Parkstone	SZ031904	Poole Harbour
14	Sterte	SZ011911	"
15	Wytch Farm	SY978858	"
16	Power Station	SZ000911	"

Table 3.13 Parameters of sediments used for investigation of K_d with sediment type

Sediment	O.S. Grid Reference	K_d (mean) (l kg^{-1})	K_{oc} (l kg^{-1})	Organics (%)	Humic Acid (µg g^{-1})	Fe (µg g^{-1})	Mn (µg g^{-1})	C.sand (%)	M.sand (%)	F.sand (%)	C.silt (%)	M.silt (%)	F.silt (%)	V.F.silt (%)	Clay (%)	F.clay (%)
Opposite Old Mine	SW803386	12357	1053	11.73	965	78005	152.4	0.21	1.94	12.53	14.27	47.60	0.81	0.11	0.18	10.70
Devoran	SW794388	2616	389	6.72	1224	49949	32.8	0.00	16.75	20.56	10.03	8.81	10.82	9.00	6.34	11.62
Old Mine	SW803387	8333	786	10.6	581	39404	216.0	0.00	0.17	5.72	31.56	39.83	2.85	0.96	0.37	6.53
Talland	SX250510	248	188	1.32	4	1092	114.9	7.30	76.27	10.06	3.61	0.37	0.27	0.24	0.21	1.23
Plym (Point Cottage)	SX508554	8166	2160	3.78	912	1927	18.4	0.12	1.47	21.23	20.44	11.33	7.72	4.74	4.60	13.32
E. Looe (Terrace Br.)	SX250510	8322	1392	5.98	4084	3413	92.5	0.28	3.58	29.69	26.64	8.87	8.87	4.70	2.03	4.97
St. John's Lake	SX441535	16699	2080	8.03	3469	7326	77.4	0.00	0.57	4.14	24.27	15.40	14.58	7.67	6.15	11.65
Calstock	SX435686	17666	1801	9.81	18179	13036	515.3	0.00	0.04	0.67	18.61	15.93	12.85	8.48	15.64	13.60
Cotehele	SX424678	17172	1823	9.42	21258	13884	569.5	0.00	0.03	1.35	9.58	15.02	12.89	9.39	14.73	21.14
Totton	SU368133	11851	1523	7.78	8733	4848	51.2	0.64	24.63	18.01	22.83	4.47	3.73	3.40	3.45	14.12
Warsash	SU489059	10767	2441	4.41	2510	4840	127.2	0.03	0.63	26.32	23.60	6.33	4.15	3.56	3.08	18.29
St. Denys	SU437139	8883	882	10.07	4945	1480	18.9	0.18	1.26	16.55	27.19	12.20	10.55	6.28	6.74	7.72
Parkstone	SZ031904	5102	1505	3.39	1139	6121	51.9	0.34	18.40	48.49	9.70	2.58	1.70	1.49	1.61	11.72
Sterte	SZ011911	24677	2814	8.77	3446	13703	185.7	0.00	0.16	1.05	23.18	15.80	14.32	23.05	1.57	3.90
Wytch Farm	SY978858	16793	1910	8.79	4999	13982	256.2	0.00	0.06	0.96	7.65	18.04	14.66	9.35	26.29	4.36
Power Station	SZ000911	20005	2470	8.1	3844	12923	85.7	0.02	0.73	4.19	21.00	14.45	35.76	2.45	0.64	3.86

Table 3.14 Correlation matrix for sediment parameters.

	Mean K_d	K_{oc}	Organics	Humic acids	Iron	Manganese	Coarse sand	Medium sand	Fine sand	Coarse silt	Medium silt	Fine silt	Very fine silt	clay	Fine clay
K_{oc}	**0.791** *p=.000*														
Organics	**0.5739** *p=.020*	0.0771 *p=.776*													
Humic acids	0.4574 *p=.075*	0.2275 *p=.397*	0.3776 *p=.149*												
Iron	-0.0515 *p=.850*	-0.3571 *p=.175*	**0.5192** *p=.039*	-0.1861 *p=.490*											
Manganese	0.4585 *p=.074*	0.1721 *p=.524*	0.4119 *p=.113*	**0.8356** *p=.000*	0.0666 *p=.806*										
Coarse sand	-0.4964 *p=.050*	**-0.5044** *p=.046*	**-0.571** *p=.021*	-0.2231 *p=.406*	-0.2089 *p=.437*	-0.1132 *p=.676*									
Medium sand	**-0.6069** *p=.013*	**-0.5775** *p=.019*	**-0.6312** *p=.009*	-0.2469 *p=.357*	-0.1751 *p=.517*	-0.2283 *p=.395*	**0.9409** *p=.000*								
Fine sand	**-0.6252** *p=.010*	-0.1825 *p=.499*	**-0.5735** *p=.020*	-0.3975 *p=.127*	-0.1467 *p=.588*	**-0.5497** *p=.027*	-0.0207 *p=.939*	0.1596 *p=.555*							
Coarse silt	0.2498 *p=.351*	0.28 *p=.294*	0.3476 *p=.187*	-0.0991 *p=.715*	-0.1236 *p=.648*	-0.2185 *p=.416*	-0.4711 *p=.065*	**-0.5292** *p=.035*	-0.0291 *p=.915*						
Medium silt	0.2592 *p=.332*	-0.0857 *p=.752*	**0.7432** *p=.001*	-0.0712 *p=.793*	**0.7794** *p=.000*	0.2382 *p=.374*	-0.3318 *p=.209*	-0.4506 *p=.080*	-0.4028 *p=.122*	0.2383 *p=.374*					
Fine silt	**0.632** *p=.009*	**0.5171** *p=.040*	0.2682 *p=.315*	0.2359 *p=.379*	-0.1667 *p=.537*	0.1199 *p=.658*	-0.3334 *p=.207*	-0.3991 *p=.126*	-0.4572 *p=.075*	0.1178 *p=.664*	-0.0611 *p=.822*				
Very fine silt	**0.6144** *p=.011*	0.4754 *p=.063*	0.2527 *p=.345*	0.2815 *p=.291*	-0.1272 *p=.639*	0.2792 *p=.295*	-0.3056 *p=.250*	-0.3307 *p=.211*	-0.4048 *p=.120*	0.0564 *p=.836*	-0.1011 *p=.709*	0.3339 *p=.206*			
clay	0.3267 *p=.217*	0.1625 *p=.548*	0.2817 *p=.291*	**0.5716** *p=.021*	-0.1272 *p=.639*	**0.5826** *p=.018*	-0.2388 *p=.373*	-0.2792 *p=.295*	-0.4048 *p=.120*	-0.3517 *p=.182*	-0.0318 *p=.907*	0.2332 *p=.385*	0.3335 *p=.207*		
Fine clay	0.0417 *p=.878*	0.2101 *p=.435*	0.0604 *p=.824*	**0.5257** *p=.036*	0.0217 *p=.936*	0.3431 *p=.193*	-0.4049 *p=.120*	-0.3123 *p=.239*	0.1769 *p=.512*	-0.019 *p=.944*	-0.0681 *p=.802*	-0.2039 *p=.449*	-0.0424 *p=.876*	0.1912 *p=.478*	

Significant correlations highlighted in red.

lower K_d value than the silts due to a combination of the lower specific surface area, and the lower organic content of the sand fraction. Karickhoff et al. (1979) noted that the functional behaviour of the sand fraction can be distinguished from that of the finer (<50μm) sediments with respect to the sorption of hydrophobic pollutants. Furthermore, it was also found that the partition coefficient (K_d) could be normalised to the organic carbon content f_{oc} (on a fractional mass basis) in the sediment:

$$K_{oc} = K_d / f_{oc}$$

and that a normally distributed dependence of K_{oc} on particle size was apparent for pyrene and methoxychlor, with a low K_{oc} for sands increasing to a maximum in the medium or fine silt, and decreasing somewhat for clay-sized particles (Karickhoff et al., 1979). K_{oc} values for the 16 sediments studied (Table 3.14), showed positive correlations with the fine and very fine silt fractions in the sediment, suggesting that a similar grainsize/organic content dependence for the TBT partition coefficient may exist.

Humic acids were associated with the clay fraction of sediments, which also showed a significant correlation to 1M-HCl extractable manganese. Correlations of K_d with the humic acid and manganese content of the sediments, although not significant (P=0.075, and 0.074 respectively) suggest that adsorption to sediment phases other than organics alone may be important for TBT. Interestingly, the poor correlation with 1N-HCl extractable Fe (r^2=-0.051) suggests that iron oxyhydroxides are not important adsorptive phases for TBT, at least at the salinity (35‰.) employed here.

It does not therefore seem possible to attribute TBT partitioning to any single phase within natural sediments, due to the covariance of these parameters, but from these results it would appear that the organic content together with the proportion of silt and clay in sediments are among the major determinants of TBT sorption. Further investigations with more sediment types, and size fractions, would be useful to extend this database for TBT partitioning, and lead to greater insight and understanding of what is clearly a complex physicochemical process.

3.4 TBT Desorption

The desorption experiments described above (Section 3.3.4.2) were all short-term (24 hour) and equilibrium between the two phases was achieved, demonstrating that sediments have the potential to act as a source of TBT as well as a sink, depending upon the relative TBT concentrations in sediments and the surrounding water.

The long-term significance of TBT contaminated sediment, and its role as a source of TBT to the overlying water has also been investigated in a long-term laboratory experiment lasting almost two years. Here, sediment which had been inoculated with ^{14}C-TBT was maintained under constant laboratory conditions (15°C, 12:12 hour light:dark) in a flow-through sea water system, such that complete exchange of overlying seawater occurred, on average, every 24 hours. Water samples were collected regularly and sediment samples taken approximately monthly to monitor the change in TBT concentrations in each phase. In addition, equilibrium TBT desorption experiments were conducted at regular intervals throughout the duration of the experiment, using the standard sorption protocol.

The results for this experiment are summarised in Table 3.15. The *in situ* concentrations of TBT and DBT in the sediment and water phases are shown in the left hand section, while the right hand section shows the mean TBT and DBT concentrations measured in water and sediment during the equilibrium desorption experiments, using sediment samples from the same experimental tank. The results of the *in situ* measurements are plotted against time in Figure 3.20 and show that over the first month or so, there was an initial period of extensive leaching of TBT from the sediment, with concentrations of up to ~50 ng l^{-1} recorded in the overlying water. After this initial period, water levels remained approximately static, at a level below 5 ng l^{-1}. Over the same time the TBT concentration in the sediment declined demonstrating that, even under flow-through conditions, sediments may indeed act as reservoirs of TBT and slowly release the contaminant back into overlying water in the absence of any obvious sediment disturbance. Obviously, these results were derived from controlled experiments, and conditions in the environment would be expected to be much more variable, nevertheless long-term release has been demonstrated to occur.

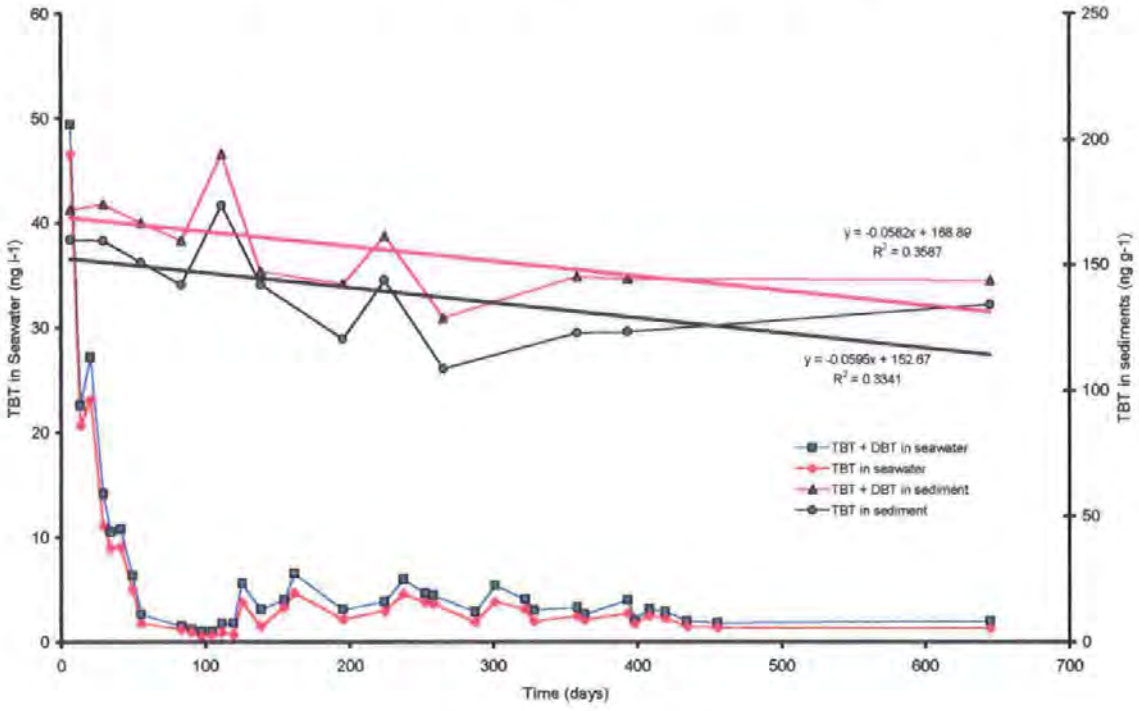
Table 3.15 Summary results for long-term TBT desorption from sediment.

	"In situ" †					Experimental ‡				
Day	TBT + DBT in water (ng l^{-1})	TBT in water (ng l^{-1})	TBT + DBT in sediment (ng g^{-1})	TBT in sediment (ng g^{-1})	Kd (l kg^{-1})	TBT + DBT in water (ng l^{-1})	TBT in water (ng l^{-1})	TBT + DBT in sediment (ng g^{-1})	TBT in sediment (ng g^{-1})	Kd (l kg^{-1})
6	49.41	46.57	171.99	159.99	3435	20.37	18.51	161.04	170.21	9197
13	22.63	20.68								
20	27.23	23.14								
29	14.16	11.16	174.16	159.72	14306	16.25	14.62	189.22	204.78	14008
34	10.53	9.01								
41	10.79	9.09								
49	6.36	5.05								
55	2.68	1.80	166.78	151.07	83979	13.50	12.06	188.45	193.89	16072
83	1.52	1.23	159.70	142.26	115214	11.97	11.07	183.05	195.90	17699
90	1.23	0.98								
97	1.04	0.63								
104	1.03	0.74								
111	1.82	0.98	194.10	173.64	176711	10.31	9.62	174.07	181.55	18863
119	1.81	0.76								
125	5.61	3.84								
138	3.12	1.55	147.42	142.16	91773	9.06	8.83	177.66	187.55	21234
154	4.08	3.38								
161	6.56	4.72								
195	3.14	2.18	142.51	120.59	55235	9.19	8.02	145.79	161.43	20140
224	3.90	3.00	161.50	143.85	47892	10.87	9.39	180.70	192.99	20549
237	6.03	4.63								
252	4.65	3.84								
258	4.49	3.66								
265			128.90	108.81		10.46	9.97	166.01	171.39	17197
287	2.94	1.96								
301	5.45	3.87								
322	4.11	3.19								
328	3.06	2.01								
358	3.32	2.50	145.45	123.13	49160					
363	2.65	2.14								
393	4.02	2.76	144.58	123.56	44771					
398	2.14	1.77								
408	3.16	2.56								
419	2.91	2.29								
434	2.04	1.52								
455	1.86	1.42								
645	2.04	1.38	143.83	134.33	97074					

† Undisturbed sediment in flow-through aquaria

‡ Standard protocol – sediment shaken for 24 h before measuring TBT + DBT in aqueous and solid phases

Figure 3.20 Total organotin and TBT concentrations in seawater and sediments during long-term desorption experiment.



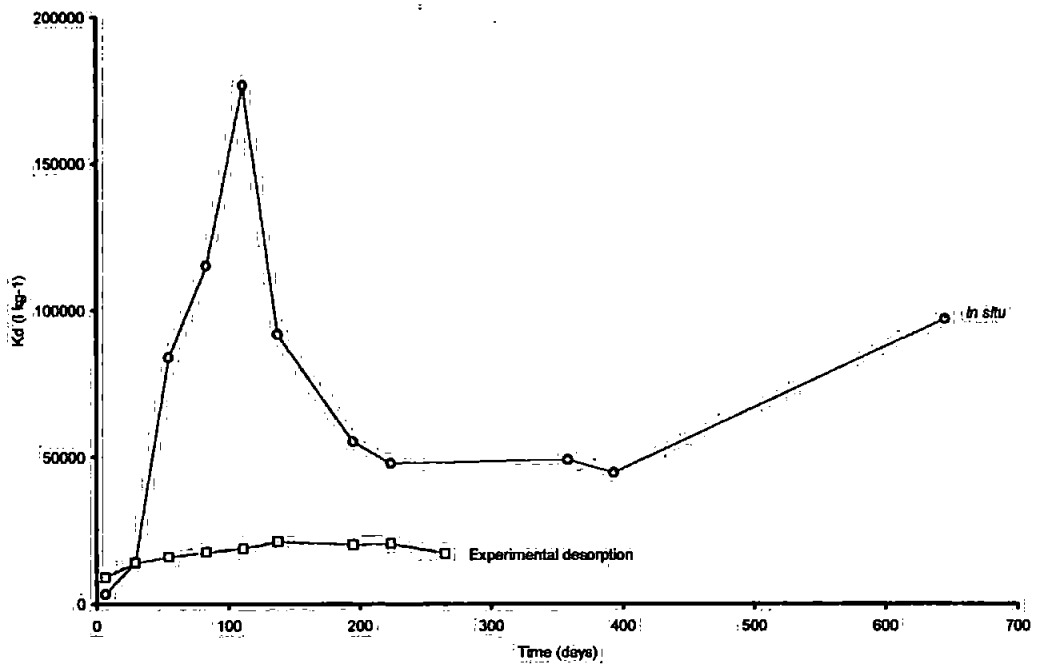
It has also been possible to provide a crude estimate the loss rate for 'total organotin' (TBT + DBT) and TBT from the sediment in this experimental system, although this was not the primary aim of this experiment. Linear regressions were applied to the data for these extracts from the sediments, and are shown together with the regression equations in Figure 3.20. Although the regression coefficients and linear trend are poor, the equations nevertheless allow very approximate values for half-lives to be calculated. Thus, solving these equations for time (x) shows that the half-life for 'total organotins' (TBT+DBT) is approximately 1450 days (nearly 4 years) while that for TBT alone is 1280 days (about 3.5 years). This slightly faster rate for TBT presumably reflects a small amount of TBT loss through degradation to DBT in addition to the loss by TBT desorption. Clearly, more carefully designed experiments over a longer timescale would be needed in order to determine these half-lives with any certainty.

It is interesting to compare the results of the *in situ* measurements with those from the equilibrium desorption experiments (Table 3.15). It can be seen that the *in situ* K_d values are considerably higher than those determined experimentally (after the initial high leach rate period) - Figure 3.21. Since the sediment TBT concentrations recorded *in situ* and

experimentally, were very similar, the high *in situ* K_d values result from the very low TBT levels recorded in the seawater of the flow through system, compared to those derived from desorption at equilibrium (vigorous shaking in batch experiments). This suggests that the sediment and water in the flow-through regime described were not in fact at equilibrium, which might be expected given that there was no sediment disturbance or re-suspension to enhance sediment/water exchange. Thus the TBT contaminated sediment in this experiment, while releasing TBT to the overlying water, did not reach its full potential as a source. Any process that results in increased sediment water mixing would therefore be expected to increase the exchange from this sediment reservoir to the water column. Such processes could include tidal re-suspension, bioturbation and in certain areas, dredging and other human disturbance. Incidental results from other experiments not reported here, but described later in this thesis, show that disturbance of surface sediments by the gastropod *Hinia reticulata* does indeed lead to higher TBT levels in the overlying water than when it is not present.

Despite the very artificial nature of this experimental system, it has nevertheless been demonstrated that sediments contaminated with TBT at environmentally realistic concentrations, may act as a source of TBT to the overlying waters for a considerable time after new inputs have ceased. Furthermore, even at sub-equilibrium levels of desorption, the concentrations of TBT leached into the overlying water (~5 ng l^{-1}) may exceed levels which have been shown to exert sub-lethal toxicity, notably to some gastropod molluscs.

Figure 3.21 Comparison of *in situ* K_d values for TBT with those derived from experimental equilibrium desorption experiments, during long-term desorption.



3.5 Summary

These investigations into the sorptive behaviour of TBT under near-natural conditions have led to several important conclusions about its likely behaviour in the environment:

1. Uptake of TBT by sediments has been shown to be best described as a logarithmic function of time. Partitioning of TBT occurs rapidly (minutes), although the achievement of equilibrium may take longer (hours) and may not necessarily be achieved in the environment.

2. The magnitude of the partition coefficient K_d is not constant, but varies with:

   a) TBT concentration. The sorption isotherm for TBT was found to follow a Freundlich-type dependency on its own concentration, with the proportion of TBT sorbed to sediments decreasing as the amount of TBT available increases. Similar behaviour has been reported for other ionizable organic solutes (Podoll and Mabey, 1987) and describe sediment-water interactions where sorption energies decrease as more energetic sites become filled This is especially important if experimental estimates of sorption behaviour, and equilibrium K_d values are to be extrapolated to natural conditions.

   b) Sediment type. This is by far the most important determinant of K_d, with measured values ranging over nearly 3 orders of magnitude, depending on sediment characteristics. Lowest values have been shown to occur primarily in sandy, low organic sediments, while the highest K_d values have been determined in organic rich sediments with a high proportion of fine silt. However, many of the physicochemical characteristics of sediments appear to be involved in TBT sorption so that no single parameter may be used to gauge the affinity of a particular sediment for TBT.

   c) Salinity and pH. Although the greatest proportion of TBT in a sediment-water system at equilibrium was adsorbed to the sediment phase under all conditions investigated, changes in the salinity and pH of the sea water phase can influence the distribution of TBT between phases. Higher proportions of dissolved TBT occur at salinities intermediate between fresh and fully marine waters. For pH, highest adsorption occurs around neutral pH and declines in both acid and alkaline waters, presumably due to pH

effects on the solubility of TBT, and the surface charge properties of the particulate phase.

d) Suspended sediment load. Although this was shown to exert no significant effect upon K_d in the current study, limited investigations across a range of levels of suspended solids suggest that at high solid loads the sediment may transfer a solute-binding material to the dissolved phase, increasing its capacity for TBT and therefore partially reducing K_d. Results suggest that this could be an important area to research in the future.

3. The potential for natural sediments to act as long-term reservoirs of TBT after new inputs have ceased, has been demonstrated under laboratory conditions. Sediment contaminated with TBT at a moderately high environmental concentration has been shown to release TBT back to the overlying water (albeit under artificial conditions) at sub-lethally toxic concentrations. This process has been shown to occur continually over many months with a probable half-time for release of the order of years. It would be expected that similarly contaminated sediments in the field would also act as sources of TBT to the overlying water. However, natural processes that enhance sediment re-suspension would be expected to increase the release rate of TBT, thus increasing the concentration of inputs from this source while also reducing the half-time for persistence in the sediment.

4 BIOACCUMULATION OF TBT BY *HINIA RETICULATA*

4.1 Introduction

Nassariids have been studied in connection with TBT pollution almost since the beginning of interest in TBT ecotoxicology. During the development of *Nucella lapillus* as an indicator for TBT contamination in the UK, it was apparent that its use would be restricted as terminally affected populations died out, and that another, perhaps less sensitive indicator species would be useful (Bryan et al., 1993b). An alternative candidate appeared to be the netted dogwhelk *Hinia reticulata* which occurs on sediment shores, overlapping to some extent with *Nucella lapillus* and therefore providing a complementary role in TBT studies (Bryan et al., 1993b). An additional advantage in the use of *Hinia reticulata* is that this species is not sterilised like *Nucella lapillus*; vas deferens tissue does not block the vulva, while a relatively long planktonic stage in the life cycle allows for recruitment into affected areas.

The effects of TBT on *Hinia reticulata*, its morphological expression of imposex and its use as a bioindicator of TBT in comparison to *Nucella lapillus* have been studied recently (Bryan et al., 1993b; Stroben et al., 1992a; Stroben et al., 1992b). As a brief summary: Bryan et al. (1993b) found no significant difference between TBT and DBT concentrations in male and female *Hinia reticulata*, although Stroben et al. (1992b) working on animals from northern France found lower levels (89% and 86% respectively) in males compared with females. The relationship between TBT concentrations in tissues and those in seawater showed concentration factors from 28,500 at 25 ng l^{-1} TBT to 74,500 at 2.5 ng l^{-1} TBT (Bryan et al., 1993b) and a regression between tissue and water concentrations showed significant tissue burdens at zero TBT in water, implying a proportion of this is derived from other sources such as food or sediment (Stroben et al., 1992a). Comparisons between *Hinia reticulata* and *Nucella lapillus*, including indices of imposex and tissue accumulation showed the vas deferens sequence (VDS) to be the most suitable index of effect, followed by relative penis length (RPL). However, since imposex is irreversible, and population imposex declines only slowly due to the longevity of this species, analysis of *Hinia reticulata* tissues were shown to provide a better indication of impact when environmental TBT levels were declining (Bryan et al., 1993b).

The aim of this group of experiments was to determine the extent to which *Hinia reticulata* is capable of bioaccumulating ^{14}C-TBT in its whole body tissue from labelled sediment and water under laboratory conditions, and, if possible to determine the relative importance of each of these sources as a route for uptake.

Initially an experiment of approximately 50 days duration was set up to simply establish whether or not TBT was accumulated via either the sediment or water phases, and to test the proposed experimental protocol.

Subsequent to this initial experiment, another series of experiments was set up to investigate uptake and depuration at a range of TBT concentrations in sediments encompassing those found in the environment, to determine whether *Hinia reticulata* is a quantitative indicator of TBT concentrations in sediments.

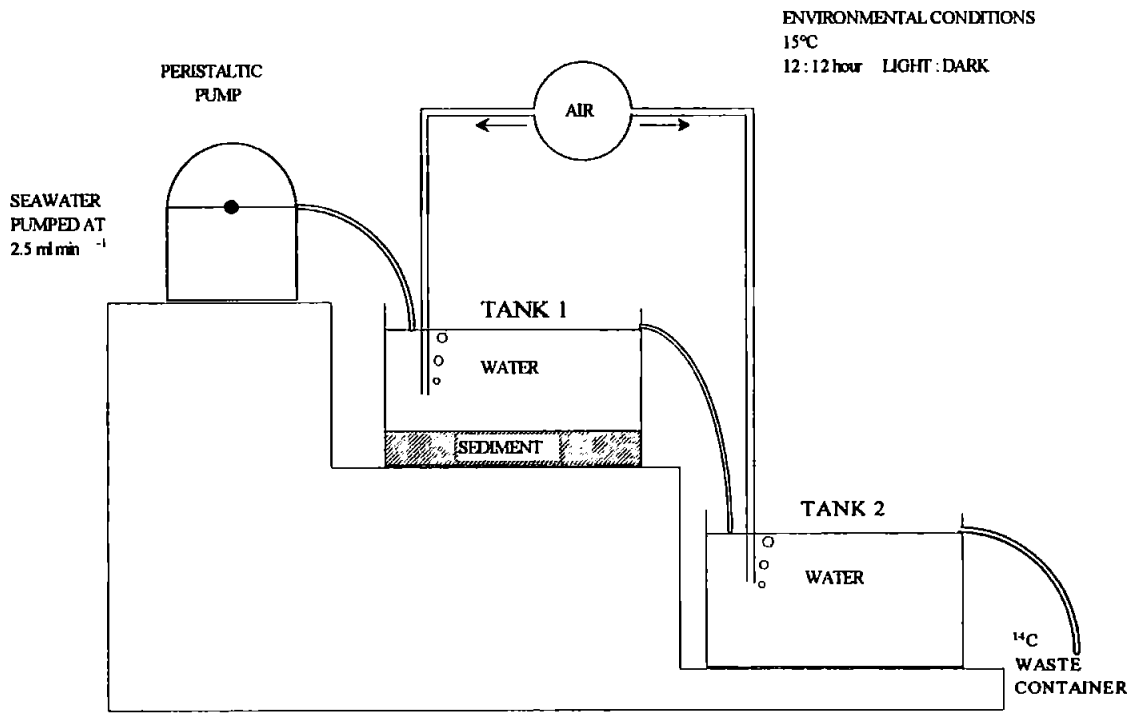
Later experiments were designed to see whether *Hinia* reduced its body burden of TBT when moved to a clean environment, and if a depuration rate could be established. In addition, a further experiment to investigate the depuration of TBT from the body tissues was conducted where the dose was injected rather than taken up by natural means.

The final experiment described in this chapter investigated whether or not the ingestion of TBT-contaminated sediment is a significant route for TBT uptake by *Hinia reticulata*.

4.2 Materials and methods

The design and layout of components were essentially identical in all experiments with *Hinia reticulata*. A flow-through aquarium system was employed (Figure 4.1), consisting of a series of experimental units; each unit comprised of two 5 litre capacity polycarbonate aquarium tanks fitted with overflow pipes and supplied with sea water from a header tank via a peristaltic pump (Watson-Marlow). The tanks were housed on a tiered rack, with the upper tank of the pair containing the ^{14}C-TBT labelled sediment, and supplied with seawater from the pump at a rate of approximately 2.5 ml min^{-1}. The overflow from this tank discharged into the second tank which contained no sediment, and which in turn discharged via its overflow into a waste water container. Both tanks were aerated by compressed air lines feeding through a manifold system and jets, which had the additional effect of creating a mixing current within the tanks.

Figure 4.1 Schematic diagram of an experimental unit used in the exposure of *Hinia reticulata* to ^{14}C-TBT labelled sediment and water.

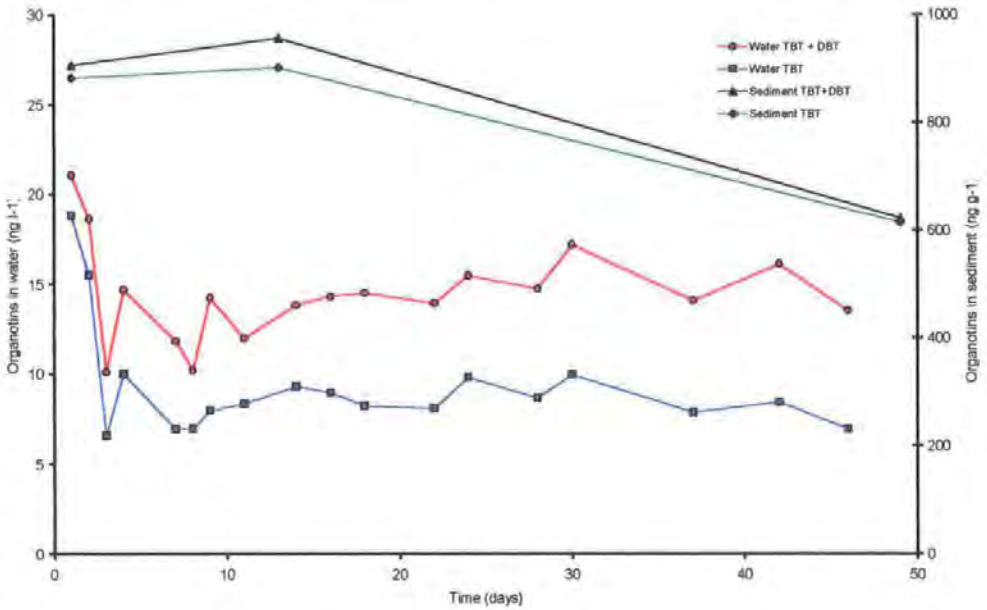


The rationale behind this design is that animals placed in the first tank were exposed to TBT bound to the sediment and also to desorbed TBT in the water, while animals in the second tank were exposed only to desorbed TBT in the water. The rate of supply of sea water was such that the residence time for each tank was approximately 24 hours, and 48 hours for the whole unit. Initial experiments showed there to be no significant difference in the mean TBT concentrations in the water of both tanks once the system had settled and equilibrated after being set up.

4.3 Uptake from sediments and water

In this 50 day experiment, experimental units were set up as described, with estuarine sediment (East Looe) that had been spiked with approximately 1 μg g^{-1} dry weight of ^{14}C-TBT, and allowed to equilibrate under flow through conditions for 1 month prior to the addition of *Hinia reticulata*. At least 60 whelks (of approximately 25 mm shell length) were added to each of the 2 experimental tanks and samples of 10 animals were removed from each after 6, 14, 21, 31 and 49 days of exposure. At each sampling interval the individuals were measured (shell height) and the soft tissues removed by carefully cracking the shell in a bench vice. Each animal was weighed, sexed and frozen in a glass vial for no more than 1 week prior to extraction and analysis for total and hexane extractable organotins (described in Chapter 2). Water and

Figure 4.2 Organotin concentrations in sediments and water during exposure of *Hinia reticulata*.



sediment samples were also analysed at regular intervals throughout the experiment. Summary results for organotin concentrations in water and sediments are presented in Table 4.1 and Figure 4.2.

TBT concentrations in the sediments ranged from approximately 900 ng g^{-1} at the beginning of the experiment when *Hinia reticulata* were added, but reduced to almost 600 ng g^{-1} after 50 days. TBT + DBT concentrations were slightly higher, but Table 4.1 shows that 94.3 – 98.8% of the total activity was present in the TBT extract. TBT+DBT concentrations in the water ranged from 21 ng l^{-1} on day 1 of the experiment down to a minimum of approximately 10 ng l^{-1} later on. 'TBT' concentrations were lower, from 18.8 ng l^{-1} at the start to a minimum of about 6 ng l^{-1}. Initial TBT concentrations in the water were slightly higher than those later on in this experiment (Figure 4.2) and were probably the result of sudden bioturbation of the sediment following the addition *Hinia reticulata* to the tanks and the time taken for complete water exchange through the system. In addition, the TBT fraction in the aqueous phase was considerably lower (51.1 – 89.2%) than in the sediments, indicating that some debutylisation of TBT occurred in the system, either in the sediment, with subsequent release of DBT to the overlying water; directly in the water column; or perhaps by processes occurring within *Hinia reticulata*.

Table 4.1 Summary results for organotins in water, sediments and *Hinia reticulata* during 50 day exposure experiment.

	Exposure concentrations						Mean organotin concentrations in *Hinia reticulata* (n = 10)							
	Water			Sediment			Water exposed				Sediment and water exposed			
Day	TBT + DBT (ng l^{-1})	TBT (ng l^{-1})	% TBT	TBT + DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT		Total (ng g^{-1})	TBT + DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT	Total (ng g^{-1})	TBT + DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
1	21.06	18.80	88.65%	906.1	882.9	97.50%									
2	18.63	15.50	82.11%												
3	10.12	6.57	64.32%												
4	14.66	10.00	67.02%												
5															
6							Mean (n=10)	32.2	27.8	25.4	78.93%	147.8	133.7	118.1	79.88%
mean	16.12	12.72	75.53%				S.D.	11.5	15.3	11.9		57.9	52.5	46.3	
7	11.83	6.92	58.32%				Bioconc. factor		1725	1999			0.148	0.134	
8	10.18	6.96	68.76%				% from water					21.79%	20.79%	21.53%	
9	14.21	7.95	57.27%				% from sediment					78.21%	79.21%	78.47%	
10															
11	11.96	8.34	69.68%												
12															
13				956.3	902.1	94.31%									
14	13.79	9.29	67.28%				Mean (n=10)	139.1	126.6	103.2	74.14%	554.8	506.6	474.9	85.59%
mean	14.05	10.04	69.27%				S.D.	67.1	52.1	44.7		146.1	136.1	126.9	
15							Bioconc. factor		9010	10278			0.530	0.526	
16	14.27	8.93	62.31%				% from water					25.08%	24.99%	21.72%	
17							% from sediment					74.92%	75.01%	78.28%	
18	14.48	8.21	56.67%												
19															
20															
21							Mean (n=10)	117.1	100.2	71.8	61.30%	545.2	476.1	423.2	77.62%
mean	14.11	9.77	67.49%				S.D.	53.7	49.0	37.9		140.6	138.2	147.3	
22	13.90	8.07	57.71%				Bioconc. factor		7100	7350			0.498	0.469	
23							% from water					21.49%	21.04%	16.97%	
24	15.44	9.80	63.05%				% from sediment					78.51%	78.96%	83.03%	
25															

Continued

Table 4.1 (continued) Summary results for organotins in water, sediments and *Hinia reticulata* during 50 day exposure experiment.

Day	Exposure concentrations						Mean organotin concentrations in *Hinia reticulata* (n = 10)							
	Water			Sediment			Water exposed					Sediment and water exposed		
	TBT + DBT ($ng\ l^{-1}$)	TBT ($ng\ l^{-1}$)	% TBT	TBT + DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT + DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT + DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
26														
27														
28	14.72	8.65	58.41%											
29														
30	17.16	9.94	57.60%											
31							Mean (n=10)	203.5	189.6	137.3	67.44%	945.5	930.8	801.3	84.75%
mean	14.43	9.60	65.28%				S.D.	80.3	90.9	68.7		167.6	147.2	164.1	
32							Bioconc. factor		13139	14305			0.973	0.888	
33							% from water					21.53%	20.36%	17.13%	
34							% from sediment					78.47%	79.64%	82.87%	
35															
36															
37	14.05	7.85	55.38%												
38															
39															
40															
41															
42	16.09	8.41	51.38%												
43															
44															
45															
46	13.52	6.91	51.07%												
47															
48															
49				622.0	614.4	98.76%	Mean (n=10)	160.1	141.6	107.0	66.83%	851.0	694.1	585.8	68.84%
mean	14.45	9.28	63.17%				S.D.	62.0	61.0	44.5		134.2	141.5	141.9	
							Bioconc. factor		9801	11527			1.116	0.953	
							% from water					18.82%	20.40%	18.27%	
							% from sediment					81.18%	79.60%	81.73%	

Combining the measured TBT concentrations in sediments and water gave an apparent sorption coefficient for the sediments, which ranged from approximately 47,000 – 97,000 l kg^{-1} during the course of the experiment. This value is considerably higher than the equilibrium K_d value (8,322 l kg^{-1}) reported earlier for this sediment, and suggests that sub-equilibrium levels of TBT were being desorbed, which might be expected given that the sediment and water were not being well mixed. Nevertheless this experiment shows that TBT desorbs from sediments under flow-through conditions (as in the long-term desorption experiment described earlier) and that bioturbation increases the concentrations of TBT released to the overlying water.

Results for organotin concentrations in *Hinia reticulata* which were exposed to labelled sediments and water, and those which were exposed to labelled water only, are also summarised in Table 4.1. Analysis of the data shows that there were highly significant differences ($P<0.0002$, ANOVA) between Total, (TBT+DBT), and TBT concentrations in *Hinia reticulata* exposed to labelled sediment, and those exposed to labelled water alone, at each sampling interval. Since the uptake profiles were very similar for 'Total', (TBT + DBT) and TBT fractions, only those for the total are shown (Figure 4.3).

Figure 4.3 Total organotin concentrations in *Hinia reticulata* exposed to ^{14}C-TBT labelled sediments and water.

(Error bars show mean *(n=10)* ± 1 S. D.)

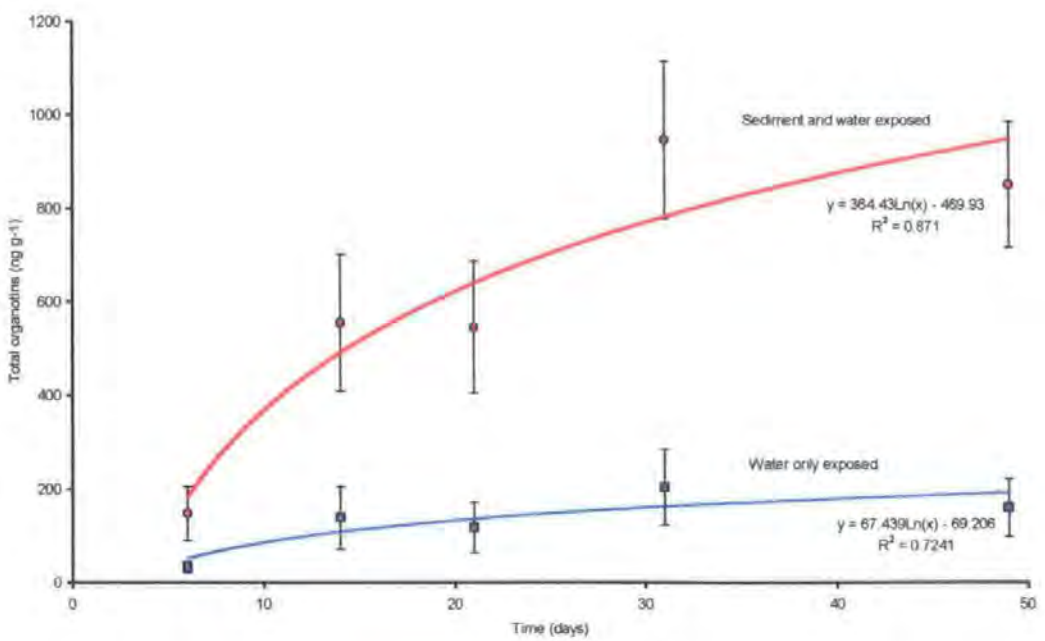


Figure 4.4 Speciation of organotins in water-exposed *Hinia reticulata*

(Columns show means *(n=10)* ± 1 S.D.)

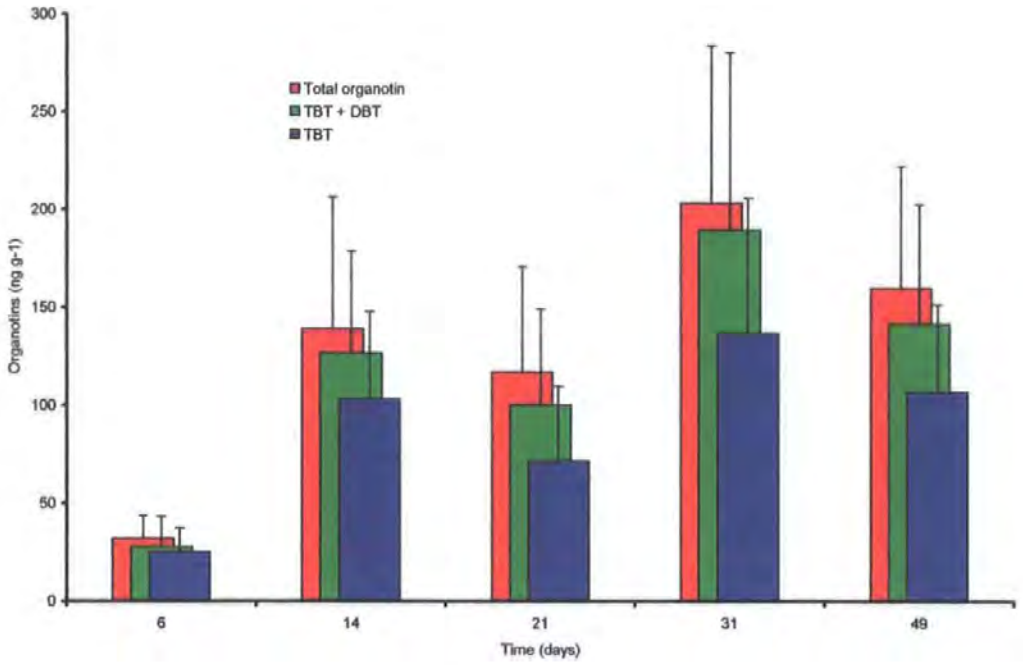
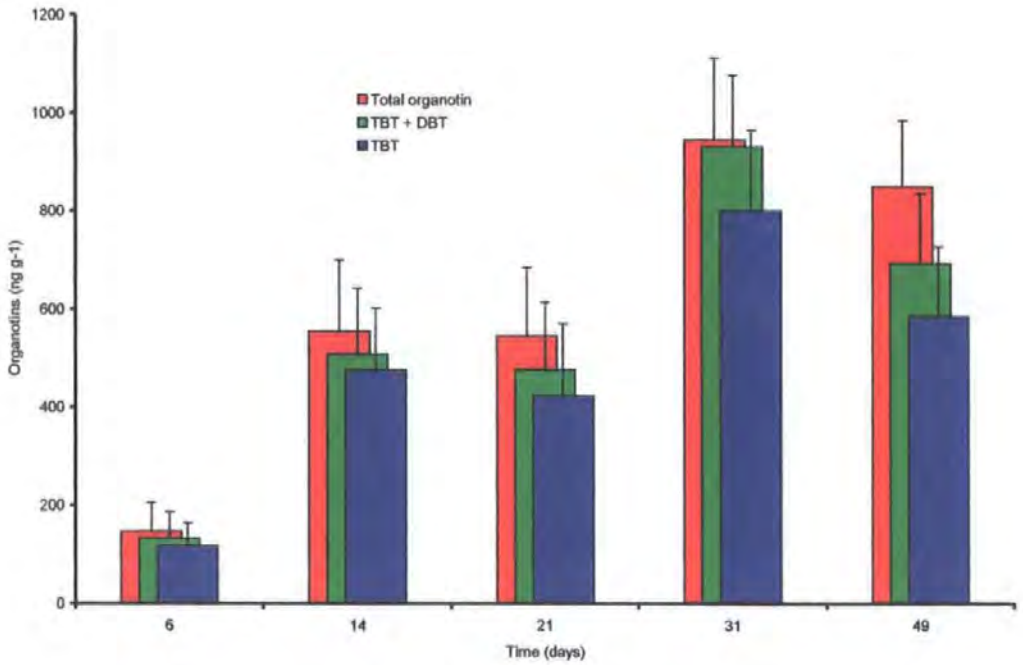


Figure 4.5 Speciation of organotins in sediment-exposed *Hinia reticulata*

(Columns show means *(n=10)* ± 1 S.D.)



Butyltin 'speciation', as determined by the extraction processes and treatments employed (see Chapter 2), shows that in both water and sediment exposed animals, the major fraction of the body burden, at all sampling intervals, appeared to be present as the tributyltin form (Figures 4.4 and 4.5). Data in Table 4.1 suggests that the proportion of TBT in sediment-exposed animals if averaged across all sampling times, was slightly higher (79.3%) than in water-exposed animals (69.7%) perhaps reflecting the higher proportion of TBT found in the sediments compared to the overlying seawater. It must be stressed however that this is only an approximation, in the absence of definitive speciation methods. These results could be explained by either of two means:

- That TBT is the major organotin species taken up by *Hinia reticulata*, and that once incorporated into the body tissues, it is not quickly metabolised, or
- That when TBT is taken up by *Hinia reticulata* it is metabolised and the products (debutylated species) are excreted.

Later experiments have been conducted with this question in mind.

By dividing the body burdens of animals (in ng g^{-1}) exposed via each phase, by the mean concentration in that phase (in ng ml^{-1} for water, and ng g^{-1} for sediment) during the period of exposure (the means shown in Table 4.1) it is possible to calculate the corresponding bioconcentration factors for *Hinia reticulata* (Table 4.1). Extracting these values from Table 4.1, the bioconcentration factors for water, and sediment exposed animals over the course of this experiment were:

Table 4.1a Bioconcentration Factors for *Hinia reticulata*

	Water-exposed †		Sediment and water exposed ‡	
Day	TBT + DBT	TBT	TBT + DBT	TBT
6	1725	1999	0.148	0.134
14	9010	10278	0.530	0.526
21	7100	7350	0.498	0.469
31	13139	14305	0.973	0.888
49	9801	11527	1.116	0.953

† = concentration in tissue / concentration in water
‡ = concentration in tissue / concentration in sediment

Thus, bioconcentration factors for TBT in seawater increase from about 2000 after 6 days exposure to 14000 after 1 month, indicating that *Hinia reticulata* is a very efficient accumulator of TBT from solution. Bioconcentration factors for TBT+DBT were slightly lower, suggesting that DBT is not bioconcentrated to the same degree as TBT. Bioconcentration factors for sediment-bound contaminants are generally lower than those for contaminants in solution, since sediment concentrations may often be several orders of magnitude higher than those in solution. Thus, the bioconcentration factors for TBT in *Hinia reticulata* exposed to sediment increase from 0.134 after 6 days to nearly 1.0 after 49 days, demonstrating that over the course of this experiment the final tissue concentration almost equalled that of the sediment. Bryan et al. (1993b) reported bioconcentration factors for TBT in *Hinia reticulata* of 30,000 at 25 ng l^{-1} TBT in seawater to 75,000 at 2.5 ng l^{-1} based on field observations in SW England while Stroben et al. (1992b) reported a BCF of 55,000 for *Hinia reticulata* from Roscoff harbour, France, which had been exposed to average water concentrations of 47 ng l^{-1} TBT. Therefore, considering the relatively short-term exposure of this laboratory experiment, final bioconcentration factors reported here appear to be in agreement with those of field exposed populations for relatively high levels of contamination.

If it is assumed that *Hinia reticulata* exposed to both sediment and water obtain approximately the same amount of their total body burden from the water, as individuals exposed to water alone, it is possible, by subtraction, to estimate the relative contributions of sediment and water as TBT sources when *Hinia reticulata* is exposed to both phases. The results of these estimations are shown in Table 4.1, and indicate that approximately 75 - 80% of the total butyltin body burden of *Hinia reticulata* may be attributed to uptake from the sediment phase, the remainder being derived indirectly from the overlying water via sediment desorption.

In summary, this experiment has shown *Hinia reticulata* to be a very efficient accumulator of TBT from solution, but when additionally exposed to sediment-bound TBT it accumulates significantly higher burdens, with approximately 75-80% attributable to the sediment phase. Most of this body burden remained as TBT, irrespective of the route of exposure, but was highest in sediment-exposed individuals, although in all cases there was some evidence of TBT debutylation.

4.4 Uptake in response to sediment TBT concentrations

The aim of this experiment was to determine whether *Hinia reticulata* is a quantitative indicator of sediment TBT contamination, and to increase the understanding of the relative importance of the sediment and water phases for uptake.

Hinia reticulata were exposed in experimental units of identical layout to those described for the previous experiment. Upper tanks contained labelled sediment, and received a peristaltic pumped seawater supply, while the lower tanks received labelled runoff water from the upper tanks.

Three parallel experimental units were set up with *Hinia reticulata* exposed to three levels of sediment contamination. Initial sediment concentrations were spiked at approximately 0.1, 1.0 and 10 μg g^{-1} dry weight TBT, although the measured levels in each tank were slightly lower (Table 4.2), which was due to an underestimate of the wet to dry weight ratio when the sediment was spiked. Sediments were left to equilibrate in the flow-through tanks for 1 month after inoculation, then 30 *Hinia reticulata* were added to each tank. Water and sediment concentrations were monitored regularly throughout the exposure period (16 and 4 times respectively), and samples of 5 *Hinia reticulata* were removed from each tank after 9, 16, 23, 30, 44 and 74 days exposure. Table 4.2 shows summary data for the water and sediment concentrations at each exposure level over the period during which *Hinia reticulata* were exposed.

Table 4.2 Summary data for water and sediment concentrations at low, medium and high levels during exposure of *Hinia reticulata*.

	Water			Sediment		
	TBT + DBT (ng l^{-1})	TBT (ng l^{-1})	% TBT	TBT + DBT (μg g^{-1})	TBT (μg g^{-1})	% TBT
			Low Level			
Mean	**2.893**	**1.963**	**67.5%**	**0.067**	**0.068**	**101.8%**
n	16	16	16	4	4	4
Std. Dev.	1.133	0.875	12.8%	0.005	0.003	2.8%
max.	4.845	3.472	95.1%	0.072	0.072	104.2%
min.	1.502	0.925	48.6%	0.063	0.065	97.8%
			Medium Level			
Mean	**19.46**	**13.30**	**68.6%**	**0.619**	**0.642**	**103.9%**
n	16	16	16	4	4	4
Std. Dev.	3.11	2.64	10.9%	0.050	0.038	2.4%
max.	25.46	18.39	103.0%	0.679	0.684	106.7%
min.	14.10	8.73	57.3%	0.559	0.597	100.7%
			High Level			
Mean	**329.9**	**213.9**	**64.8%**	**5.906**	**6.124**	**103.7%**
n	16	16	16	4	4	4
Std. Dev.	74.44	56.18	7.1%	0.449	0.382	2.1%
max.	493.6	362.17	77.8%	6.227	6.332	105.7%
min.	210.1	134.77	54.0%	5.248	5.551	101.6%

Therefore, the low and medium level exposures may be taken as representative of field concentrations which have been found in many UK estuaries over the last decade, while the high level exposure concentrations were representative of some of the most highly contaminated marina areas, reviewed in chapter 1. Comparison of 'total' (TBT + DBT) concentrations in seawater and the TBT fraction shows that at all exposure levels an average of 64 – 69% was present as TBT, indicating that some debutylisation of TBT occurred within the system, as was found in the previous experiment. Sediment 'total' and TBT concentrations at each level were very similar implying little debutylisation in the sediment phase.

Apparent sorption coefficients were also calculated using the mean sediment and water TBT concentrations at each exposure level, and were approximately 35,000; 48,000 and 29,000 l kg^{-1} for low, medium and high levels respectively. These values again exceeded the equilibrium K_d values determined by experiment (reported in chapter 3) and indicate that the concentrations of TBT which had desorbed to overlying waters were below the equilibrium level.

Data for 'total' organotins (Optisolv extractable); TBT + DBT (hexane extractable) and TBT (NaOH washed hexane extract) in *Hinia reticulata* at each exposure level and at each sampling time are presented in Appendix 1.

Initial inspection of these results showed that as a result of the sediment concentrations used, the levels in the respective overlying waters, and the tissues of *Hinia reticulata* increased between treatments by approximately an order of magnitude. Thus, the effects of the exposure levels were multiplicative rather than additive, and as a result, variations about the mean at each level also varied by orders of magnitude so that the variances were unequal. Although ANOVA is robust enough to perform well, even if data deviate slightly from the requirements of normality, homoscedasticity and additivity, severe deviations such as these can lead to spurious conclusions. Log_{10} transformation of multiplicative data 'corrects' it to additive data and the heteroscedasticity is removed (Zar, 1984), allowing normal ANOVA techniques and multiple comparisons to be made. Thus, all comparative statistics on these results have been conducted on log_{10} transformed data. Two way analyses of variance (2-way ANOVA) were performed for 'total', 'TBT + DBT' and 'TBT' to examine the effects of exposure level (high, medium and low) and exposure time (9, 16, 23, 30, 44 and 74 days) on accumulated body

burdens in *Hinia reticulata*. The results, together with Tukey *post-hoc* comparison matrices are presented in Appendix 2.

Two-way analysis of variance (2-way ANOVA) showed that exposure level (high, medium and low) and exposure time, had significant effects upon the concentrations of butyltins accumulated in the whole body tissues of *Hinia reticulata*. Animals which were exposed to low, medium and high concentrations of TBT in sediments accumulated significantly different ($P<0.0002$, ANOVA) burdens of total, and hexane extractable butyltins at every sampling interval. These differences were apparent for both sediment-exposed animals, and for those which had been exposed to dissolved TBT alone (Appendix 2).

In all cases, uptake was non-linear, and best described as a logarithmic function of exposure time (Figures 4.6, 4.7 & 4.8) as in the previous experiment. Furthermore, the slope functions of these uptake curves increase by approximately an order of magnitude between low, medium and high level exposures, showing that accumulation in *Hinia reticulata* tissues is in proportion to the levels of exposure, which also increased by a factor of approximately 10.

Figure 4.6 Mean total butyltin concentrations in *Hinia reticulata* exposed to low levels of TBT in sediment and water.

(Error bars show mean ± 1 S.D.)

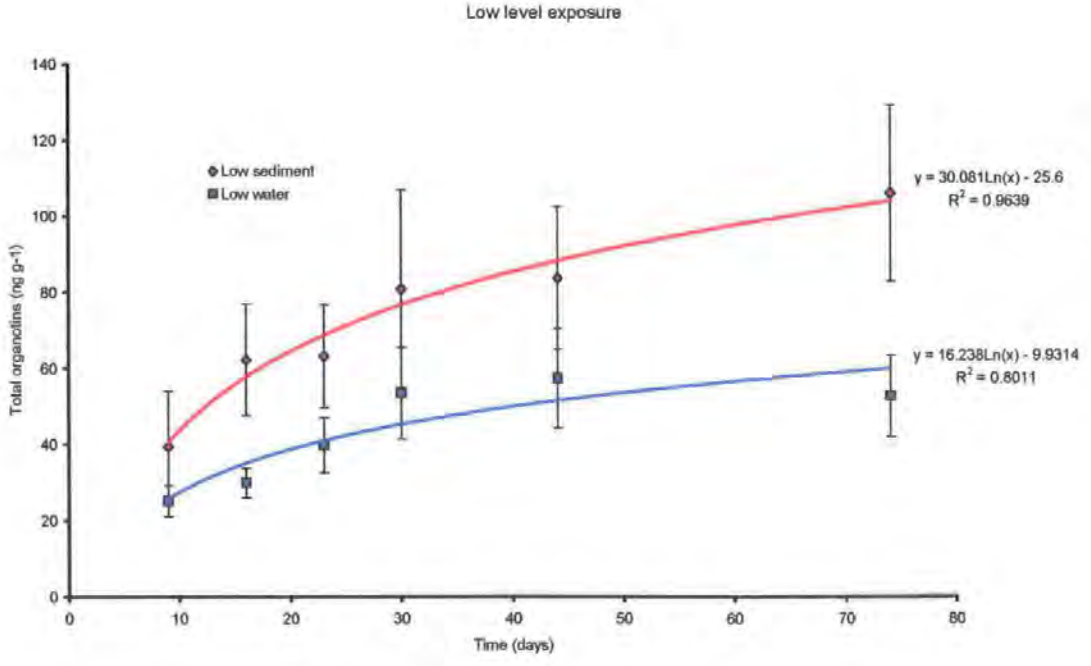


Figure 4.7 Mean total butyltin concentrations in *Hinia reticulata* exposed to medium levels of TBT in sediment and water.

(Error bars show mean ± 1 S.D.)

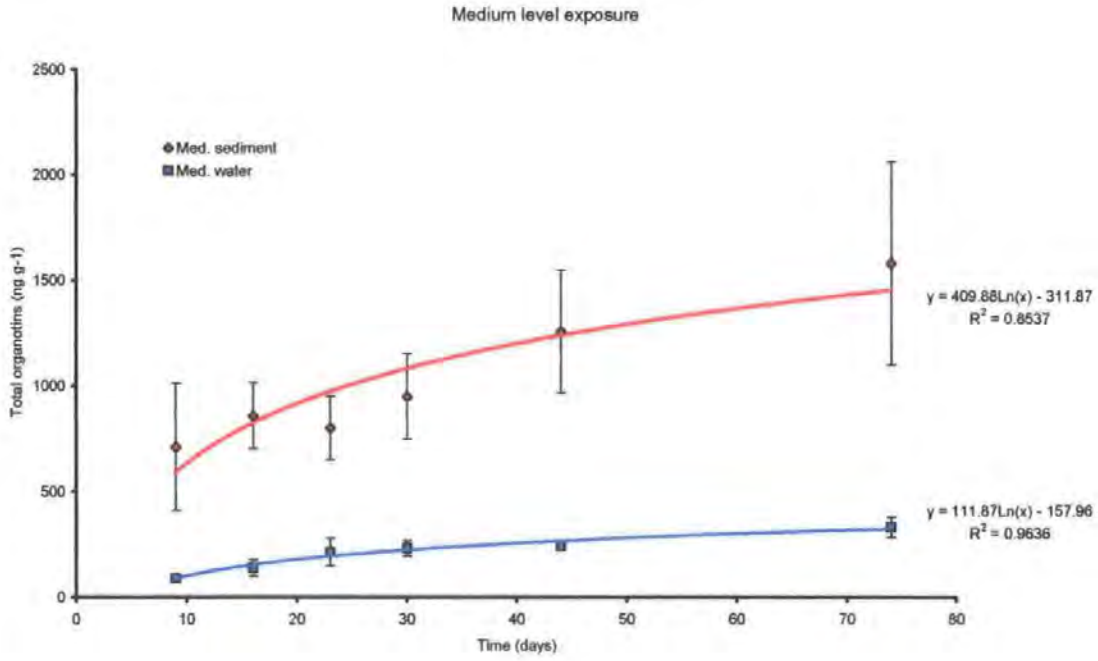
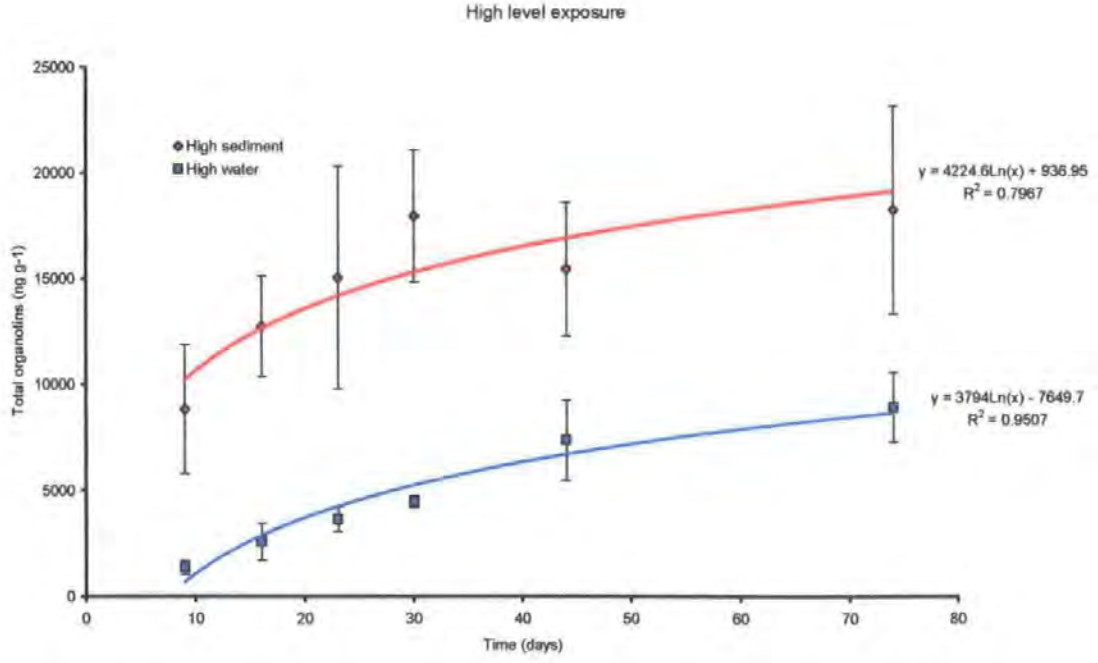


Figure 4.8 Mean total butyltin concentrations in *Hinia reticulata* exposed to high levels of TBT in sediment and water.

(Error bars show mean ± 1 S.D.)



However, within each exposure regime, differences in bioaccumulation from sediment and water were not always so clear, particularly at the low-level exposure. If 'total' (Optisolv extractable) tissue concentrations are considered: At the low exposure level (approximately 2.9 ng l^{-1} TBT + DBT in water, and 67 ng g^{-1} in sediments) there were no significant differences in accumulated burdens between sediment-exposed and water-exposed individuals after 9, 23, 30 or 44 days. Only on days 16 and 74 were the concentrations in sediment-exposed animals significantly higher ($P<0.001$) than in water-only exposed animals (Figure 4.6).

At medium level exposure (19.5 ng l^{-1} TBT+DBT in water and 620 ng g^{-1} in sediment) and high level exposure (330 ng l^{-1} TBT+DBT in water and 6000 ng g^{-1} in sediment) however, sediment-exposed animals showed significantly higher tissue concentrations of total butyltins than those exposed to water alone, at every sampling interval (Figure 4.7 & 4.8).

If it is again assumed that animals exposed to sediments and water accumulate the same total burden from the overlying water as those exposed to water alone, it is possible to estimate the proportion of the total body burden derived from the sediment, and water phases, at each sampling interval (Table 4.3).

Table 4.3 Estimated 'total' butyltin concentrations in *Hinia reticulata* tissues derived from sediments and seawater at low, medium and high exposure levels.

Day	Low		Medium		High	
	Water ng g^{-1}	Sediment ng g^{-1}	Water ng g^{-1}	Sediment ng g^{-1}	Water ng g^{-1}	Sediment ng g^{-1}
9	25.16 (63.68%)	14.35 (36.32%)	89.2 (12.54%)	622.2 (87.46%)	1380.8 (15.59%)	7474.7 (84.41%)
16	29.97 (48.09%)	32.36 (51.91%)	137.2 (16.00%)	720.5 (84.00%)	2598.9 (20.35%)	10170.7 (79.65%)
23	39.80 (62.90%)	23.47 (37.10%)	213.2 (26.60%)	588.3 (73.40%)	3641.7 (24.17%)	11427.2 (75.83%)
30	53.50 (66.08%)	27.46 (33.92%)	231.5 (24.34%)	719.6 (75.66%)	4479.6 (24.93%)	13492.3 (75.07%)
44	57.37 (68.51%)	26.38 (31.49%)	242.3 (19.27%)	1015.2 (80.73%)	7393.8 (47.77%)	8083.6 (52.23%)
74	52.78 (49.79%)	53.23 (50.21%)	330.7 (20.92%)	1250.0 (79.08%)	8948.2 (49.01%)	9310.4 (50.99%)

(Figures in parentheses represent the percentage of the total burden)

Thus, at low level exposure it would appear that only 31 - 50% of the body burden of 'total' butyltins is derived from the sediment, while 51 – 87% is accumulated from the sediment phase at medium or high exposure. Conversely it may be said that at low level exposure, TBT in the water column may be of greater importance as an uptake vector than sediment-associated TBT.

Figures 4.9 & 4.10 show the mean TBT bioconcentration factors for water-exposed and sediment-exposed animals over the course of this experiment. As in the previous experiment it appears that at all exposure levels *Hinia reticulata* is a much more effective accumulator of TBT from seawater than from sediments, although as already mentioned, this is often common due to the much higher concentrations of contaminants frequently found in sediments compared to sea water. However, the mean bioconcentration factors for sediment-exposed *Hinia reticulata* increase with increasing sediment-TBT concentrations: 1.11, 1.83 and 2.39 at low, medium and high levels respectively. Thus, although *Hinia reticulata* is an efficient accumulator of TBT from seawater, when it is additionally exposed to TBT contaminated sediments, these form an important direct source of the total burden, and their significance increases with the degree of sediment contamination.

As discussed previously, speciation of butyltins in this investigation have been approximate and operationally defined, based on different extraction techniques and treatments rather than true chemical separation techniques. Nevertheless, comparison of butyltin species within each of the exposure regimes and at each exposure level shows some interesting trends (Figure 4.11). Analysis of variance for the proportion of TBT (as a fraction of the 'total') in each treatment, with exposure time, shows that for all treatments there were significant changes ($P<0.003$, ANOVA) in the proportion of TBT in *Hinia reticulata*. An exception was the high level exposure to sediments (Figure 32) where no significant change in the proportion of TBT with time occurred ($P=0.512$, ANOVA). Speculatively, this may indicate inhibition of TBT metabolism at very high exposure levels. Further analysis of data showed there to be a significant difference in the proportion of TBT in sediment exposed and water-exposed animals across all levels ($P=0.0002$, ANOVA) with a mean of 69.1% TBT in sediment exposed *Hinia reticulata* and 61.8% TBT in those exposed only to the overlying water. This probably reflects the high

proportion of TBT in the sediments (approximately 100% TBT) compared to lower proportions in the overlying water (approximately 60 – 70% TBT) resulting from sediment desorption.

Figure 4.9 TBT bioconcentration factors for *Hinia reticulata* exposed to low, medium and high TBT levels in seawater.

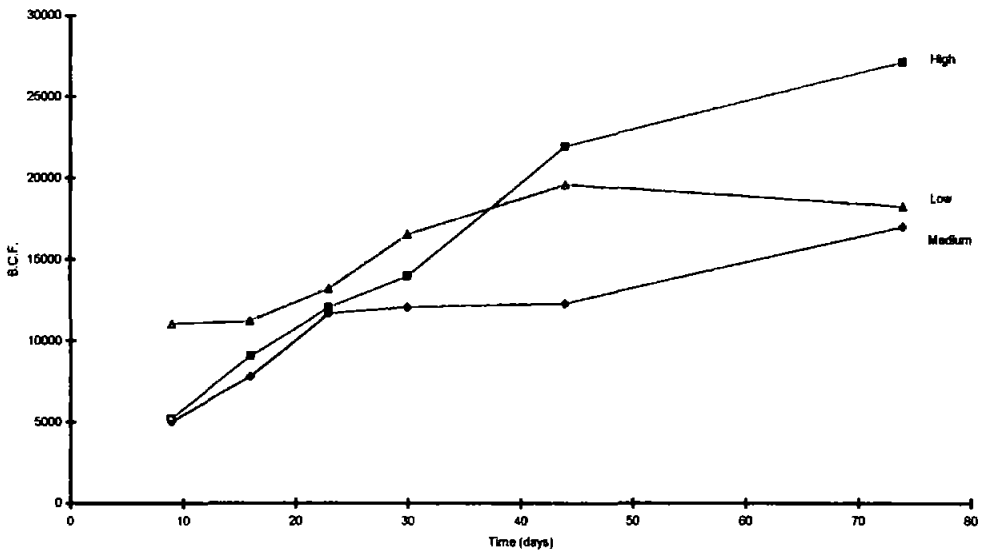


Figure 4.10 TBT bioconcentration factors for *Hinia reticulata* exposed to low, medium and high TBT levels in sediment.

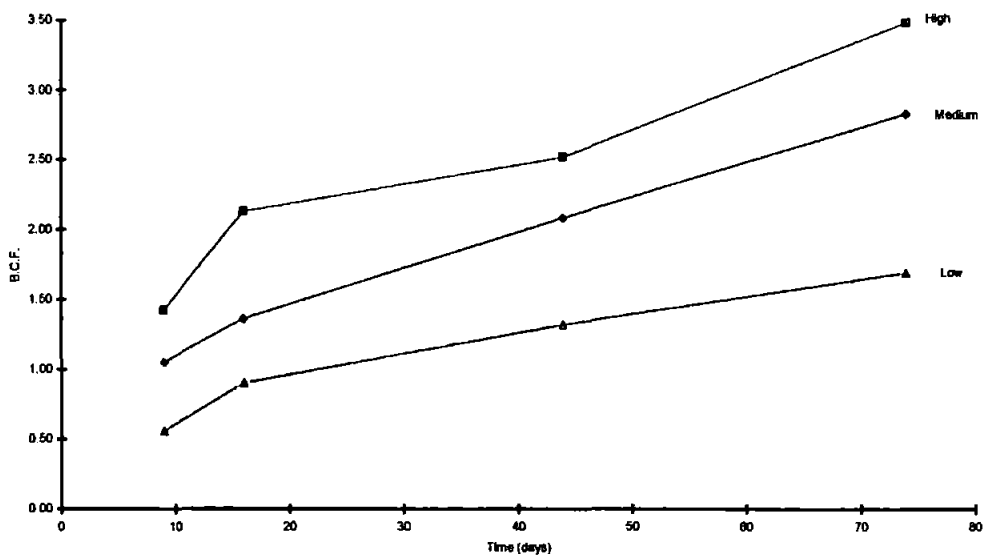
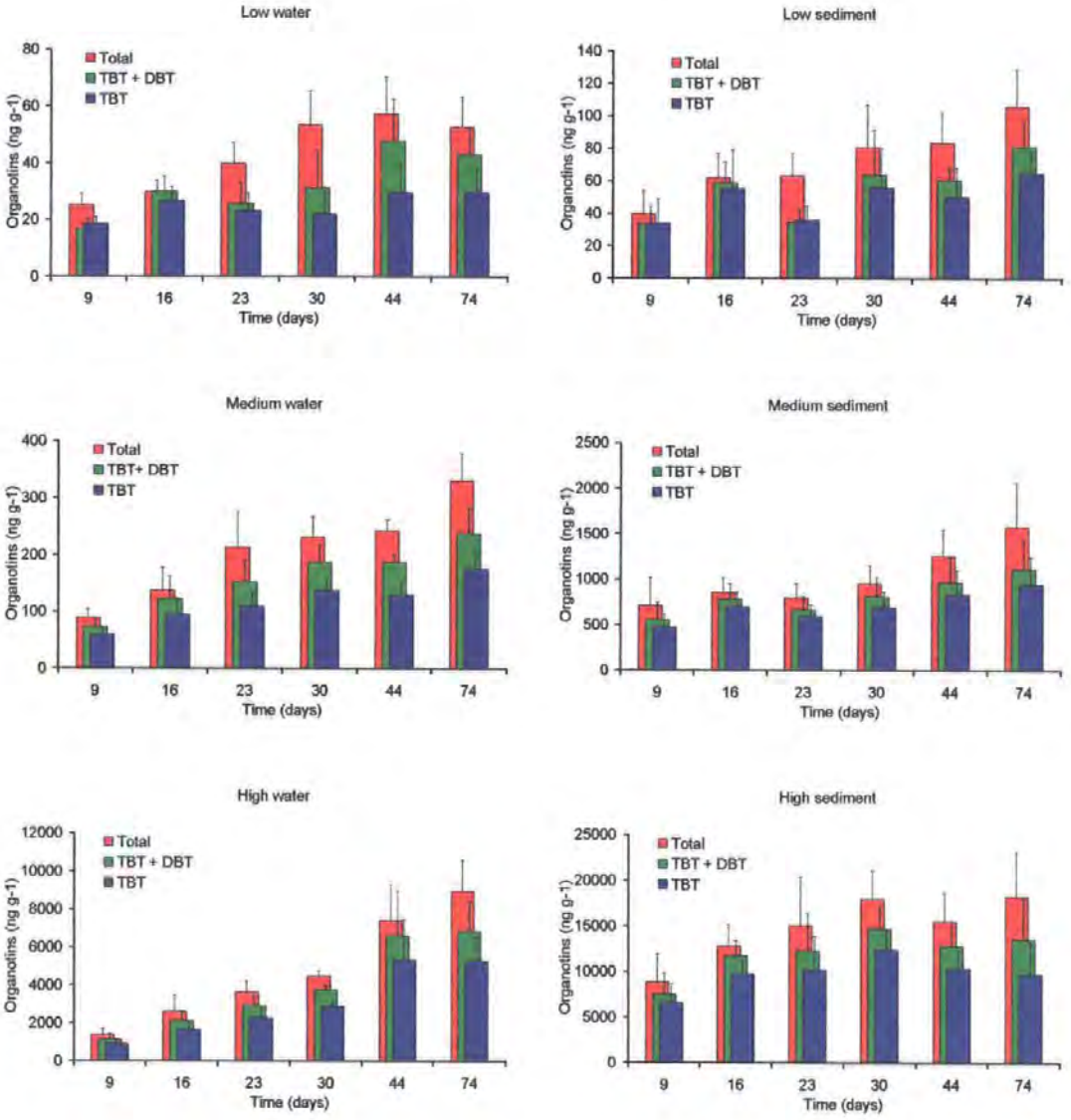


Figure 4.11 Butyltin speciation in *Hinia reticulata* exposed to low, medium and high levels of TBT in sediments and seawater.

(Columns show means (*n*=5) + 1 S.D.)



4.5 Depuration of TBT by *Hinia reticulata*

4.5.1 Depuration following uptake from sediments and water

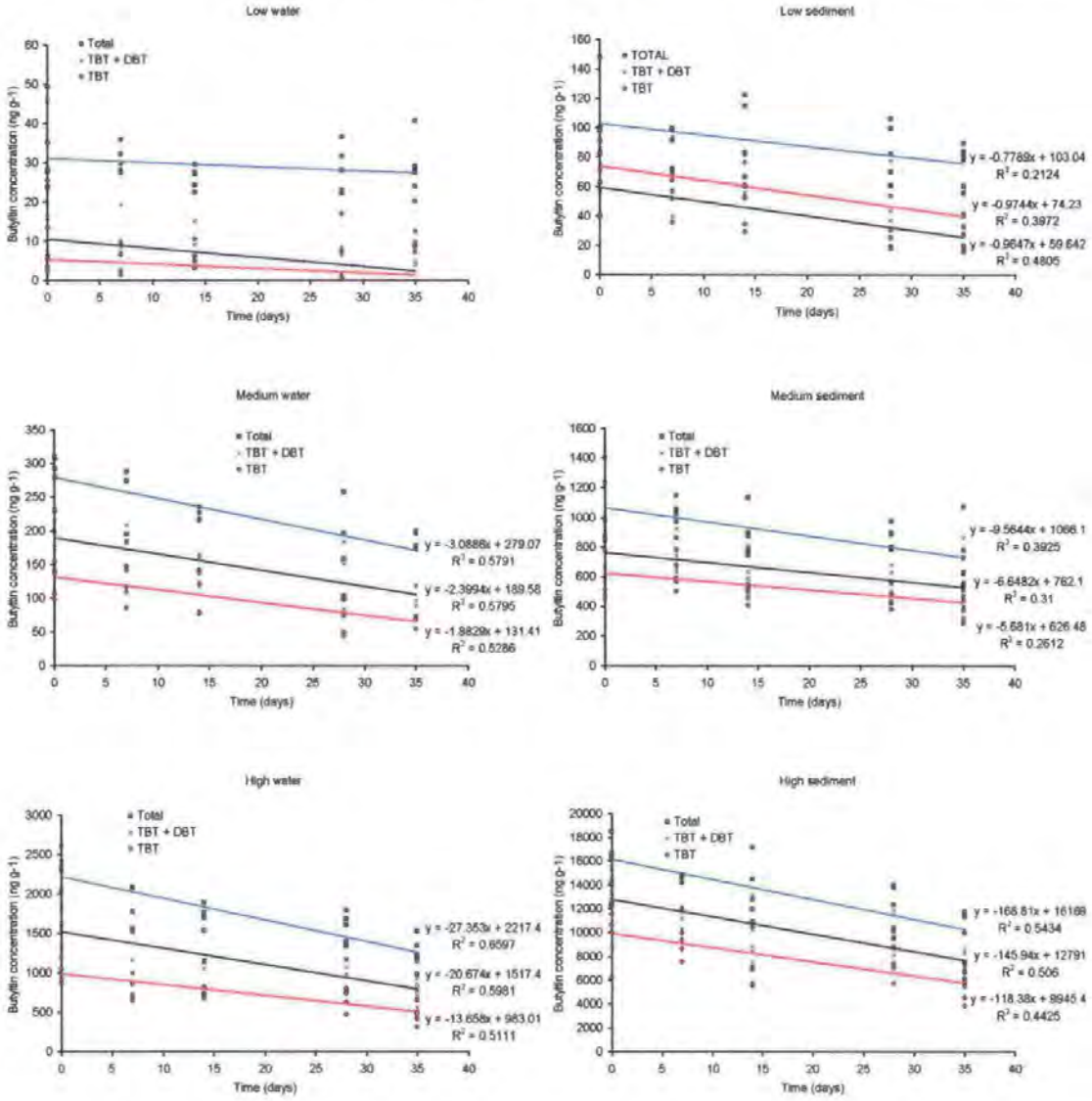
In this experiment *Hinia reticulata* were exposed to low, medium and high TBT concentrations in sediments, and seawater, under similar conditions to those described for the uptake experiment at these exposure levels. Exposure concentrations at each level are summarised in Table 4.4, and exposure lasted for 70 days, after which time all animals were removed from the exposure tanks and placed in nylon mesh cages in continuously flowing clean sea water. Samples of 5 individuals from each exposure level (high, medium and low) and exposure route (sediment and water, and water-only) were taken after 0, 7, 14, 28 and 35 days depuration and the concentrations of total and hexane extractable butyltins in each individuals whole body tissues were measured. The results are presented in Appendix 3 and summarised graphically in Figure 4.12.

Table 4.4 Mean concentrations in sediments and water at which *Hinia reticulata* were exposed prior to depuration.

(*n=6* for water and *n=3* for sediments. S.D. shown in parentheses)

Exposure	Water			Sediment		
	TBT+DBT ($ng\ l^{-1}$)	TBT ($ng\ l^{-1}$)	% TBT	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
Low	0.984 (0.214)	0.616 (0.125)	63.5% (11.0%)	0.055 (0.003)	0.058 (0.004)	105.3% (1.2%)
Medium	15.59 (3.48)	10.10 (2.25)	64.9% (2.8%)	0.551 (0.034)	0.575 (0.041)	104.5% (1.6%)
High	124.73 (35.75)	63.49 (11.90)	52.8% (8.9%)	4.838 (0.426)	5.038 (0.505)	104.1% (1.3%)

Figure 4.12 Depuration of butyltins from *Hinia reticulata* exposed to TBT in sediments and water, and TBT in water only



Analyses of the data show that in all cases except for low-level, water-exposed *Hinia reticulata* there were highly significant ($P<0.018$, ANOVA) reductions in the concentrations of 'total', 'TBT + DBT' and 'TBT' fractions during the depuration period. Figure 4.12 shows these reductions in each case, which follow approximately linear trends, although the scatter of individual data points was wide at every sampling interval due to the difference in TBT concentrations taken up by individuals during the exposure period. Consequently the correlation coefficients for linear regression are not especially high (Table 4.5).

However, from the regression equations shown in Figure 4.12 estimates of the half-times for depuration were calculated in each case (Table 4.5). Furthermore, by averaging the half-times for 'Total', 'TBT + DBT' and 'TBT' across all exposure routes and levels, it is possible to give a

gross estimate of half-times for these species in *Hinia reticulata*. Thus the mean depuration half-life for the 'total' (Optisolv extractable) concentration was 51 days; 'TBT + DBT' (hexane extractable) was 43 days; and 'TBT' (NaOH washed hexane extract) was 40 days (Table 4.5). Since the half-life of a compound is determined by two factors - loss and decay, it would appear from the decreasing half-lives in the order 'Total' > 'TBT + DBT' > 'TBT' (i.e. 'TBT' is being lost faster than the 'Total' of all butyltin species) that there is some decay (debutylisation) of TBT in *Hinia reticulata* during depuration.

Table 4.5 Estimated half-times for depuration of butyltins from *Hinia reticulata*

Exposure Route	Exposure Level	Estimated half-times (days)		
		Total	TBT + DBT	TBT
Sediment	Low	R^2 = -0.46 **66.1** P = 0.0178	R^2 = -0.63 **38.1** P = 0.00056	R^2 = -0.69 **30.9** P = 0.00009
	Medium	R^2 = -0.63 **55.7** P = 0.0006	R^2 = -0.56 **57.3** P = 0.0031	R^2 = -0.51 **55.1** P = 0.0076
	High	R^2 = -0.74 **47.9** P = 0.00002	R^2 = -0.71 **43.8** P = 0.00005	R^2 = -0.06 **42.0** P = 0.0002
Water	Low	**N.S.**	**N.S.**	**N.S.**
	Medium	R^2 = -0.76 **45.2** P = 0.00002	R^2 = -0.76 **39.5** P = 0.00002	R^2 = -0.73 **34.9** P = 0.00006
	High	R^2 = -0.81 **40.5** P<0.00001	R^2 = -0.77 **36.7** P<0.00001	R^2 = -0.71 **36.0** P = 0.00004
Mean half-life		**51.08**	**43.08**	**39.79**

4.5.2 Depuration following injection of ^{14}C-TBT into *Hinia reticulata*

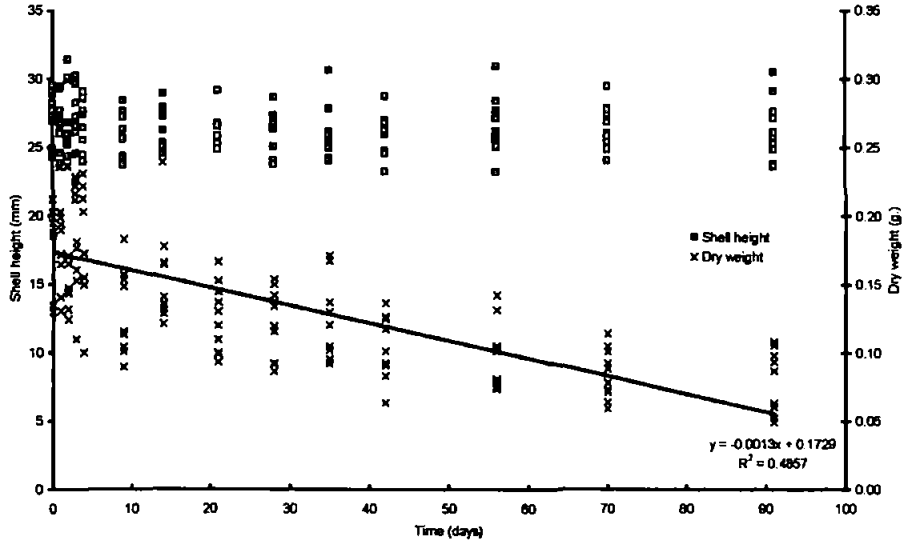
Having established these estimates of the half-lives for depuration of butyltins, it was decided to conduct a similar experiment using animals that had been dosed to an identical initial level, in order to reduce the variability seen in this experiment. Consequently, 150 individual *Hinia reticulata* of 26.8mm mean shell length (1.8mm S.D.) were anaesthetised in 75 g l^{-1} magnesium chloride and injected with 150 ng ^{14}C-TBT (1 µL) into the pedal muscle using a Hamilton micro-syringe. All animals were placed in flowing sea water at 15°C and 12:12hrs light:dark, and samples of 10 individuals removed at 14 intervals up to 3 months after injection. Thus the

experimental period was well in excess of the previously estimated half time and would therefore be expected to provide a more accurate estimation of the depuration rate.

The results for butyltin concentrations in tissue are presented in Appendix 4.

Analyses of these results show that during the depuration period there were highly significant changes in the concentrations of 'total', 'TBT + DBT' and 'TBT' fractions in *Hinia reticulata* (all $P<1x10^{-6}$, ANOVA). However, although there was no significant change in the mean size (shell height) of animals sampled throughout the experimental period (P=0.724, ANOVA) there was a highly significant decrease in the mean dry weight of animals with depuration time ($P<1x10^{-6}$, ANOVA). These changes (Figure 4.13) were expected over the long depuration period since *Hinia reticulata* were maintained, unfed, in flowing seawater. It was decided at the start not to feed *Hinia reticulata*, since increases in body mass due to feeding would effectively 'dilute' the TBT burden. Conversely, starvation effectively 'concentrated' TBT in the tissues due to a reduction in body mass, although it should be stressed that *Hinia reticulata* is capable of withstanding long periods (up to 5 months) without food (Tallmark, 1980). Consequently body burdens of the butyltin species (concentration multiplied by body mass) have been employed in this experiment, which have the additional advantage that the original amount of ^{14}C-TBT added (150 ng) was known, and provides a better initial reference point than when uptake was by natural processes.

Figure 4.13 Shell heights and whole soft-tissue dry weights of *Hinia reticulata* during depuration after injection of TBT



Depuration of 'total', 'TBT + DBT' and 'TBT' burdens followed exponential paths with the greatest rate of loss during the first few days after exposure (Figure 4.14). Estimates of the half life for loss of these butyltin species can be determined from the equations of these graphs:

At time *t*: $M = M_o e^{-\lambda t}$

where M is the mass of the compound remaining at time t, and M_o is the mass at time = 0. λ is the rate constant for depuration.

Therefore: $\frac{M_o}{2} = M_o e^{-\lambda t_{\frac{1}{2}}}$

$\log_e\left(\frac{1}{2}\right) = -\lambda t_{\frac{1}{2}}$ and since $\log_e\left(\frac{1}{2}\right) = -\log_e(2)$

$t_{\frac{1}{2}} = \frac{\log_e 2}{\lambda}$ values for λ are shown in the exponential equations fitted to data (Figure 4.14)

Thus, estimates of depuration rates based upon body burdens were 26, 25.7 and 17.9 days respectively for 'Total', 'TBT + DBT' and 'TBT'. These values are considerably shorter than those based upon the previous experiment where TBT was taken up by normal means, but the much lower variability in this data probably provides a better estimate of depuration rate.

Log_e transformed values for body burdens decrease linearly with time, the slopes in each case describing the depuration rates for the different butyltin species (Figure 4.15). This shows clearly that the loss rate for 'TBT' is higher than that for 'TBT+DBT' and 'Total butyltins', suggesting that there is additional 'loss' of TBT through debutylation.

Figure 4.14 Depuration of 'total', 'TBT + DBT' and TBT from whole tissues of *Hinia reticulata* following injection of ^{14}C-labelled TBT

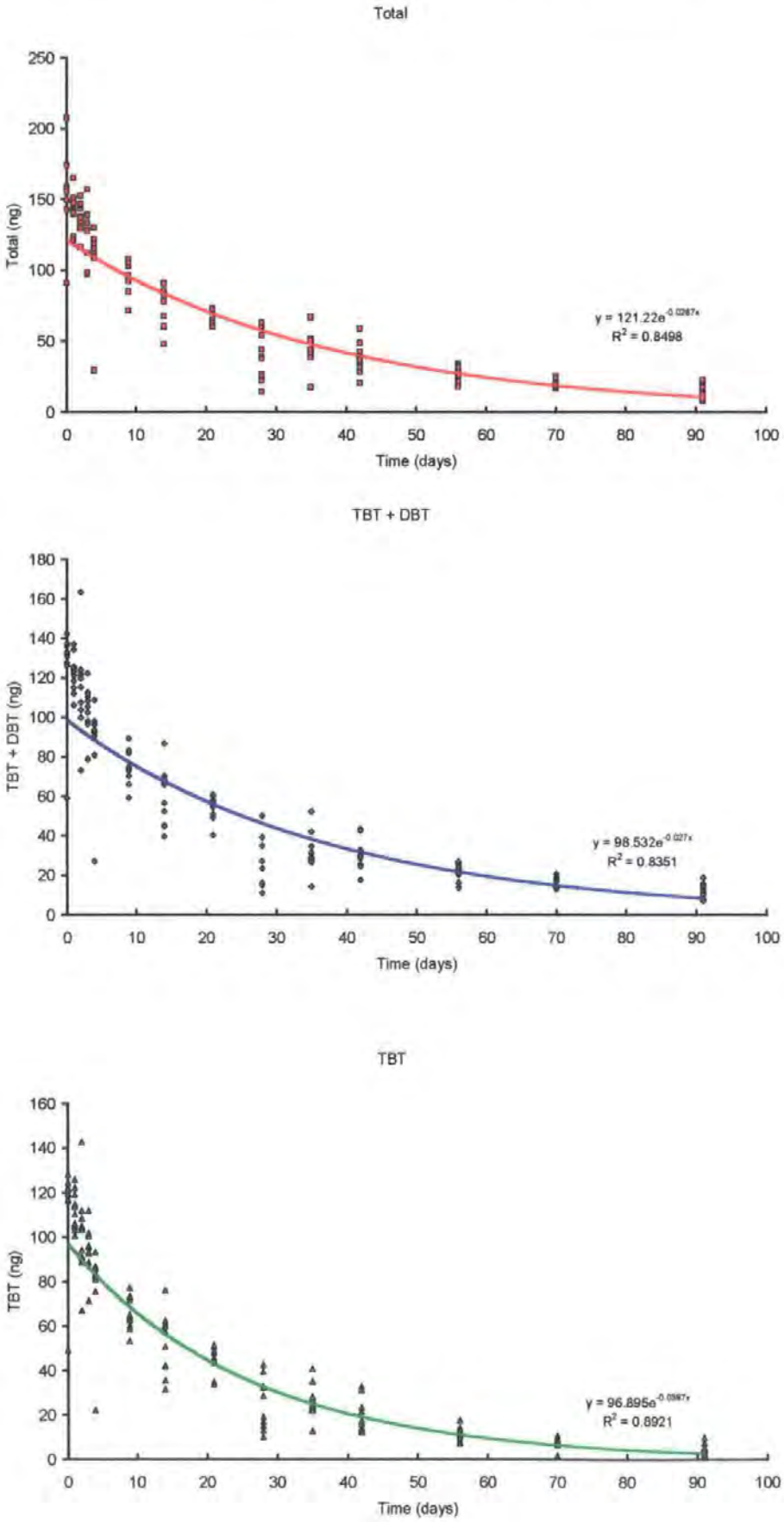


Figure 4.15 Depuration rates of 'Total', 'TBT + DBT' and 'TBT' body burdens from *Hinia reticulata* following injection of ^{14}C-labelled TBT.

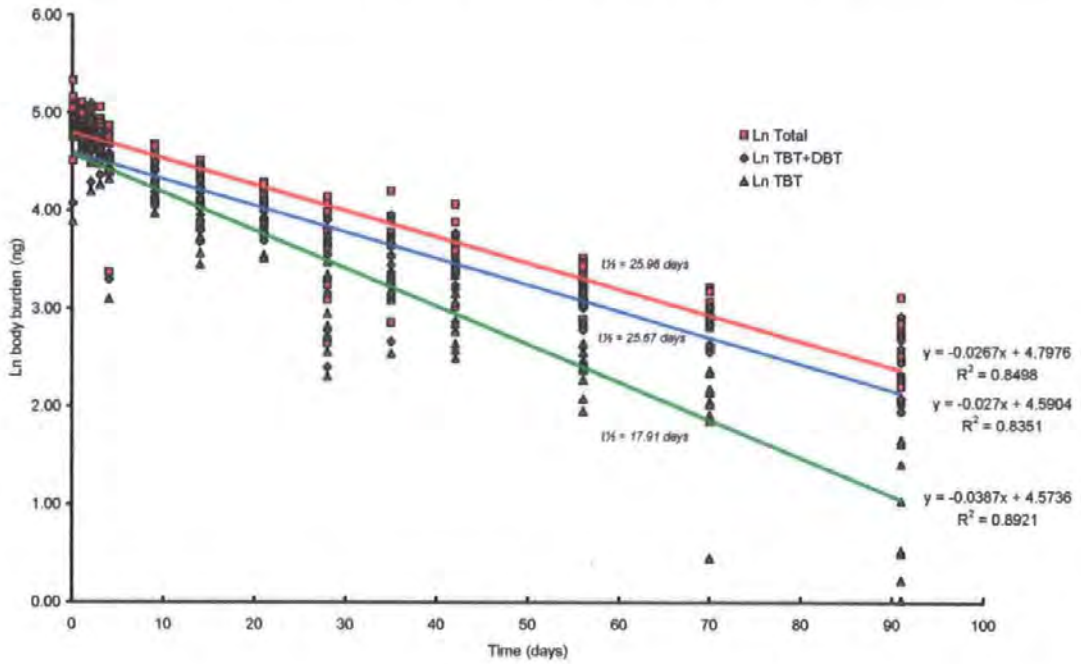


Figure 4.16 Approximate speciation of butyltins in *Hinia reticulata* during depuration, showing the relative proportions of the 'TBT' fraction.

(Error bars show mean %TBT, *n=10*, ± 1 S.D.)

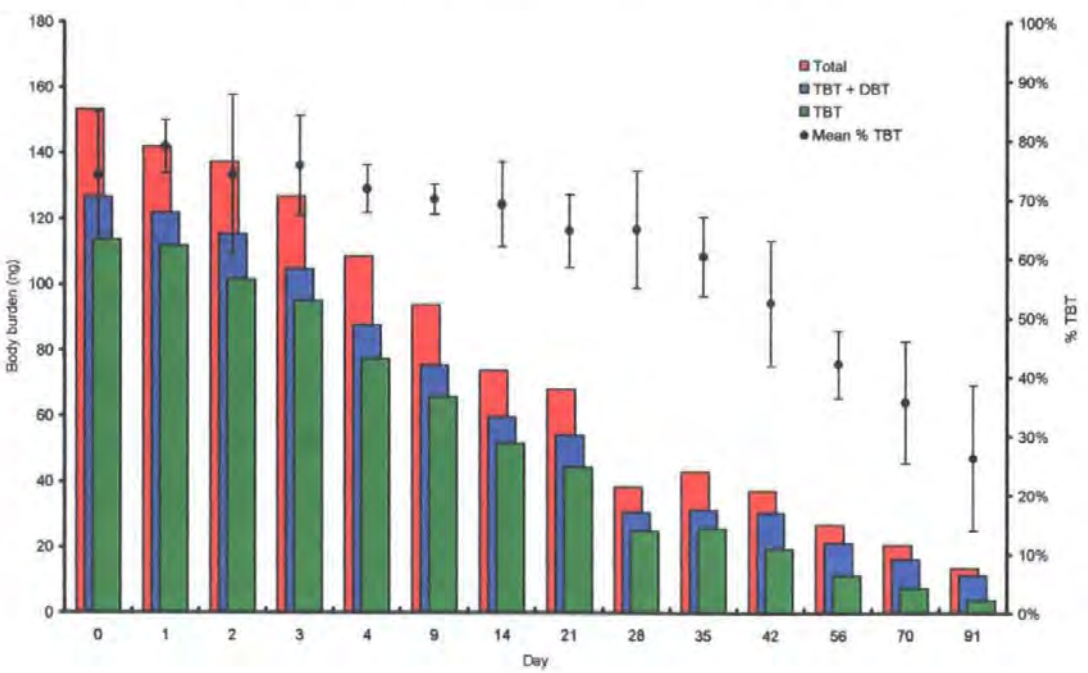


Figure 4.16 shows the body burdens of butyltin species in *Hinia reticulata* throughout the depuration period, together with the 'TBT' fraction as a percentage of the 'Total'. Analysis of these data shows there to be a highly significant reduction ($P<0.0001$, ANOVA) in the 'TBT' fraction as a proportion of the total with time, confirming that metabolism of the TBT species occurs within *Hinia reticulata*.

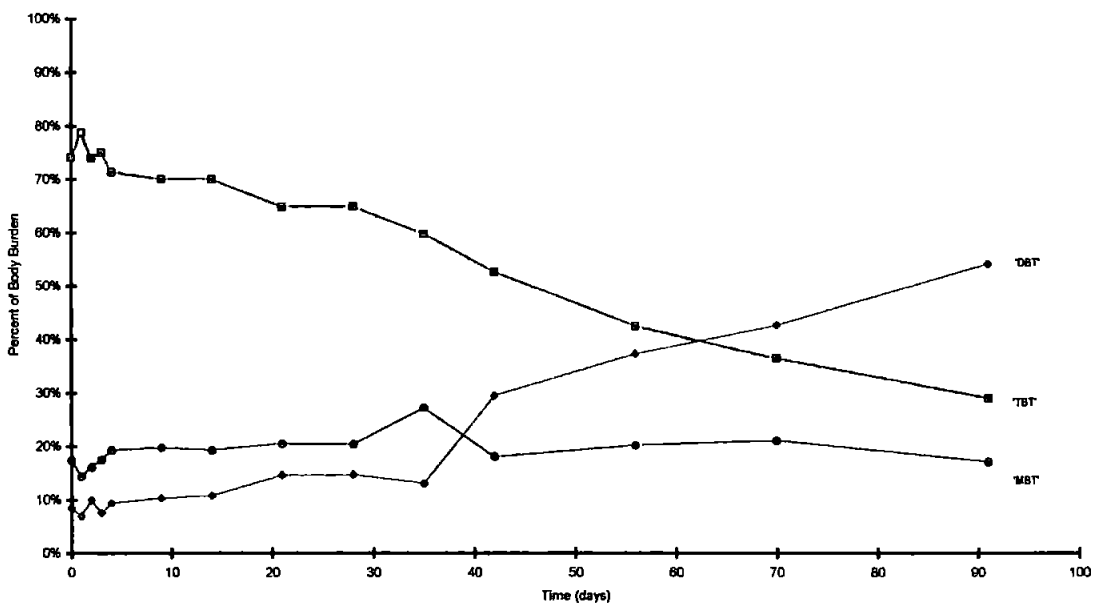
Utilising the differential extraction of butyltins by the methods employed here, it is possible to estimate the proportions of TBT, DBT and MBT as percentages of the total body burden (Table 4.6). These estimates are approximate, and probably overestimate the 'MBT' fraction which may in fact be better described as 'undefined ^{14}C' or 'non-hexane extractable butyltins' since it could include a range of metabolic products, but perhaps provides a best estimate in the absence of true speciation techniques. Nevertheless it is clear that while the total burden decreases with time from the 150 ng TBT initially injected, there is also a change in the relative proportions of the butylated species (Figure 4.17). The proportion of TBT decreases with time, while that of DBT increases. The estimated proportion of 'MBT' remains relatively constant at around 20%.

In summary, it appears that TBT is lost from the body tissues of *Hinia reticulata* with a half-life of 2 - 4 weeks, and that during this period there is some debutylisation of the tributyl species to DBT and MBT.

Table 4.6 Approximate butyltin speciation in *Hinia reticulata* showing estimated percentages of TBT, DBT and MBT.

	TOTAL (ng)		TBT + DBT (ng)		TBT (ng)		TBT	DBT	MBT
Day	Mean	*Std Dev*	Mean	*Std Dev*	Mean	*Std Dev*	%	%	%
0	153.4	*28.9*	126.8	*24.4*	113.7	*23.0*	74.1%	8.5%	17.4%
1	142.2	*12.7*	121.8	*9.5*	112.0	*8.5*	78.8%	6.9%	14.3%
2	137.3	*10.4*	115.2	*22.9*	101.6	*19.4*	74.0%	9.9%	16.1%
3	126.9	*19.2*	104.7	*11.8*	95.1	*10.6*	75.0%	7.5%	17.5%
4	108.6	*29.0*	87.7	*22.4*	77.5	*19.9*	71.3%	9.4%	19.3%
9	94.0	*11.4*	75.5	*9.0*	65.8	*7.6*	70.0%	10.3%	19.7%
14	73.9	*14.5*	59.7	*14.6*	51.7	*13.9*	70.0%	10.8%	19.3%
21	68.3	*5.0*	54.3	*6.2*	44.3	*6.0*	64.9%	14.6%	20.5%
28	38.5	*16.6*	30.6	*14.2*	25.0	*11.6*	64.9%	14.7%	20.4%
35	43.2	*12.4*	31.5	*10.1*	25.8	*7.6*	59.8%	13.1%	27.2%
42	37.5	*10.6*	30.7	*7.9*	19.7	*7.3*	52.6%	29.4%	18.0%
56	27.0	*5.1*	21.6	*4.0*	11.5	*3.0*	42.5%	37.3%	20.2%
70	21.0	*3.2*	16.6	*2.8*	7.7	*2.6*	36.4%	42.6%	21.0%
91	13.9	*4.7*	11.5	*4.0*	4.0	*2.9*	28.9%	54.0%	17.1%

Figure 4.17 Estimated proportions of TBT, DBT and MBT in *Hinia reticulata* during depuration



4.6 Uptake by ingestion of sediment

This short-term experiment was designed to investigate whether *Hinia reticulata* exposed to sediments obtain a proportion of their total body burden of TBT by direct ingestion of sediment particles. The method employed involved physically preventing ingestion in some animals, by ligaturing the proboscis, while allowing them to remain in contact with the sediment, thus enabling investigation of the importance of contact with the substrate. Ligaturing was achieved by placing the experimental animals in a 5 litre tank of seawater, partitioned with a 2mm mesh size plastic screen, and adding food (crushed *Mytilus edulis*) on the opposite side of the screen to the whelks. This caused a pronounced feeding response in *Hinia reticulata*, which quickly moved towards the food and extended their probosces through the mesh partition. Seawater in the tank was then exchanged with a solution of 75 g l^{-1} $MgCl_2$ in distilled water, which narcotised the animals with their probosces extended. Animals were removed one at a time, and under a dissecting microscope the proboscis was ligatured towards its anterior end using a Surgeons, or Ligature Knot, tied using suture silk. Animals that had been treated in this way were marked to denote their ligatured status, and were returned to clean, flowing seawater to recover.

Both ligatured and normal individuals were then exposed, in the same experimental tanks, to ^{14}C-TBT labelled sediment (approximately 7µg g^{-1} TBT dry weight). This high level dose was employed since the experiment was short-term, and greater accumulated levels would be easier to measure with reasonable precision. In addition, a second group of ligatured and normal individuals were exposed to labelled water alone (mean = 33 ng l^{-1} TBT) to determine the difference between uptake from sediments and water. Exposure via all routes lasted for two weeks with samples of 10 individuals of each type (ligatured and normal) being removed from each treatment (sediment, and water) after 7 and 15 days.

The full results are presented in Appendix 5 and a data summary is shown in Table 4.7. There were no significant differences in the mean size or dry weight of animals in each treatment ($P>0.05$, ANOVA).

3-way analysis of variance was performed on these data to investigate the effect of ligaturing, exposure route, and exposure time on the concentrations of 'total' and hexane-extractable

butyltins in whole soft tissues of *Hinia reticulata*. This showed that while the exposure route (sediment and water, or water-only) resulted in highly significant differences in tissue concentrations (P=0.000125, ANOVA), there were no significant differences (P>0.05, ANOVA) between concentrations in normal and ligatured animals exposed to the same conditions in either sea water or sediment (Figure 4.18). One exception was the concentration of hexane extractable (TBT+DBT) in sediment-exposed animals on day 15, where the mean concentration in ligatured animals (2335.8 ng g^{-1} TBT+DBT) was significantly lower (P=0.031, ANOVA) than in normal animals (2753.8 ng g^{-1} TBT+DBT).

Table 4.7 Butyltin concentrations in *Hinia reticulata* with normal or ligatured proboscis, exposed to TBT in sediments and/or water.

(Mean values shown (*n=10*), *std. dev.* in parentheses)

Exposure Route	Day	Ligatured/Normal	Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
Sediment	7	Ligatured	1703.0 *(345.9)*	1374.0 *(295.2)*	1251.5 *(288.1)*	73.3% *(4.3%)*
		Normal	1803.0 *(313.7)*	1482.0 *(311.4)*	1321.9 *(269.6)*	73.2% *(5.6%)*
Water	7	Ligatured	159.6 *(41.6)*	121.0 *(39.6)*	88.0 *(33.7)*	54.0% *(8.1%)*
		Normal	139.9 *(31.2)*	98.6 *(27.3)*	70.8 *(23.6)*	49.9% *(7.4%)*
Sediment	15	Ligatured	2997.6 *(554.0)*	2335.8 *(462.5)*	2027.0 *(444.0)*	67.3% *(4.7%)*
		Normal	3397.0 *(442.1)*	2753.8 *(442.9)*	2392.0 *(499.4)*	69.8% *(7.6%)*
Water	15	Ligatured	321.5 *(75.6)*	238.2 *(67.5)*	172.1 *(59.9)*	52.7% *(6.8%)*
		Normal	343.9 *(80.5)*	263.4 *(60.2)*	178.1 *(48.3)*	51.5% *(3.6%)*

Analysis of the proportion of TBT in each exposure regime showed highly significant differences (P=0.000116, ANOVA) in the percentage of TBT in sediment-exposed *Hinia reticulata* (70.9%) compared with those exposed to water alone (52.0%).

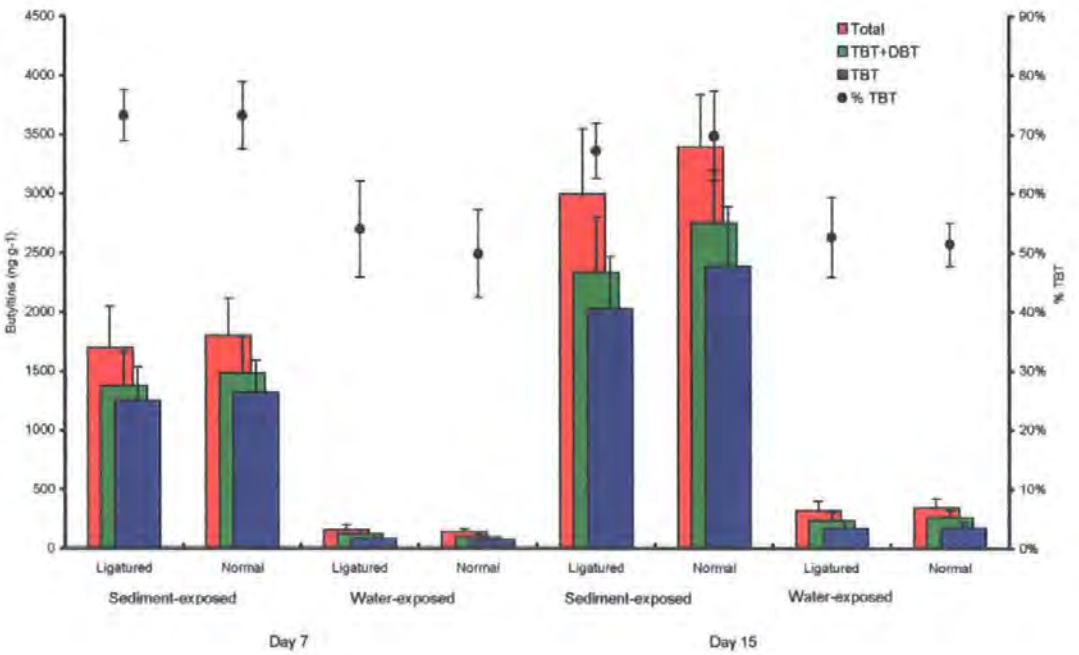
These results suggest that direct ingestion of contaminated sediment is not the major route for TBT uptake by *Hinia reticulata*, although the mean concentrations of all butyltin extracts were always slightly lower in ligatured animals exposed to sediments than in those with a normal

proboscis. Therefore although ingestion does not appear to be significant in these short-term experiments, it may possibly contribute over a longer timescale. However, in all animals dissected as part of this, and other experiments, there has been no observation of sediment particles in either the digestive tract, or in acid digests of tissues during extraction of TBT.

If it is again assumed that individuals exposed to sediments receive the same burdens from the water as those exposed to the water alone, it can be seen from Table 4.7 that approximately 90% of the total TBT present is derived from the sediment. Since ingestion does not appear to be the means of uptake, other routes - possibly related to the intimate contact of animals with sediments and pore-waters may be responsible for these elevated burdens.

Figure 4.18 Butyltin concentrations in normal and ligatured *Hinia reticulata* exposed to ^{14}C-TBT labelled sediments and water.

(Data show means ± 1 S.D.)



4.7 Discussion

Bioaccumulation is a complex process, involving transfer of a contaminant between the environment and the organism of interest, balanced against loss of the compound and/or its metabolic products from the tissues. It is this accumulated tissue burden of the contaminant, which is the feature most often measured as part of field monitoring studies. Many factors influence bioaccumulation including exposure concentration; exposure route (from water, food or sediment) together with the physicochemical form of the contaminant (charged, neutral or complexed) and the ability of the organism to metabolise and excrete the chemical and its breakdown products. A further complication in the case of TBT is that it exhibits characteristics of both organic and inorganic compounds due to the organic ligands attached to the tin atom, and the chemical speciation reactions of the tin atom itself.

The series of experiments described so far have demonstrated several important features of the bioaccumulation of sediment-bound TBT by *Hinia reticulata*, and its potential as an indicator for TBT in sediments. Firstly, all uptake experiments have shown that, far from being a medium for the sequestration and degradation of TBT, sediments can act as a significant source of TBT to benthic organisms. The bioavailability of sediment-bound TBT may be via either or both of two routes: TBT may be released back into the overlying water by sediment desorption, as already demonstrated, (which may also be enhanced by the bioturbatory effects of *Hinia reticulata*); and TBT may also be accumulated from the sediments. However, evidence from these experiments shows that in *Hinia reticulata* uptake from sediments does not appear to be by direct ingestion of the contaminated sediment particles (at least in the short-term) but may be via exposure to interstitial waters and perhaps also by direct, intimate contact of the soft tissues with the sediment. This is not surprising, since it has already been reported (Tallmark, 1980) that larger *Hinia reticulata*, such as those used in these experiments, prefer large carrion as a primary food source, while smaller individuals appear to prefer organic rich sediment as a source of detritus. In contrast, a predominantly particulate uptake route for sediment-bound TBT has been reported for the deposit-feeding clam *Scrobicularia plana* (Langston and Burt, 1991).

Comparison of TBT uptake from sediments and water by *Hinia reticulata* show that it is a very efficient accumulator of dissolved TBT, with a bioconcentration factor of approximately 14,000

after 1 months exposure to ~10 ng l^{-1} TBT in seawater. This value is similar to previously reported bioconcentration factors of 30,000 – 75,000 (Bryan et al., 1993b) although it was acknowledged that these values may have been overestimates, since a proportion of the TBT burden in these field samples may have been derived from dietary uptake. Dietary uptake by *Hinia reticulata* has already been shown to be an important vector for TBT accumulation (Stroben et al., 1992a).

Bioaccumulation from sediments was not so 'efficient' as from water, on a 'dimensionless' numerical basis due to the very much higher TBT concentrations in sediments compared with the overlying water. Nevertheless, "bioconcentration" factors approached a value of 1 after 49 days exposure to an average concentration of ~600 ng g^{-1} TBT in sediments, with an estimated 75 - 80% of the total body burden being derived from the sediment rather than the overlying water.

Various models have been developed to predict the bioaccumulation of hydrophobic chemicals, based on their partitioning behaviour in an octanol/water system and allow estimation of the bioconcentration factors (BCF's) for a wide range of chemicals (Mackay, 1982). However, using data for TBT a BCF of <500 has been predicted (Laughlin, 1996) suggesting that simple partitioning into tissues is not the only mechanism for accumulation, and that some binding process may be involved. Electrostatic or covalent bonding is known to be important for metals which form ions in seawater, while TBT solubility has been shown to vary markedly with pH (Maguire et al., 1983) indicating that the electronic structure of Sn in TBT is influenced by the chemical environment of aqueous solution, and that a TBT cation occurs in water as well as uncharged non-polar species. Therefore during bioaccumulation TBT may possess the reactive properties of a metal cation as well as the hydrophobic character of an organic compound. Maguire et al. (1983) referred to the tributyltin cation as the major species in freshwater, while Laughlin et al. (1986b) suggested that at the pH of seawater TBT may exist as the hydroxide, chloride or carbonate, and that significant variation of the TBT octanol/water partition coefficient with salinity supported the idea that chemical speciation equilibria were displaced from the TBT cation (Laughlin et al., 1986b). Evidence suggests that uptake of uncharged TBT

across epithelia or membranes appears to be the predominant uptake mechanism (Laughlin, 1996).

Bioaccumulation rates are closely correlated with uptake mechanisms. Simple partitioning is the most rapid, dependent on free-energy changes as a solute is transferred from water to a hydrophobic phase, and in single cells is limited only by diffusion rates. In macro-organisms however, bioaccumulation is controlled by the kinetics of diffusion between different tissue compartments and is therefore slower, mediated by factors such as gill irrigation and blood flow rates. A steady state of bioaccumulation therefore takes longer to achieve, and has not often been seen for organisms exposed to TBT which Laughlin (1996) regards as strong circumstantial evidence for TBT binding in tissues.

Exposure of *Hinia reticulata* to TBT concentrations in sediments and water, representative of the range of environmental concentrations that have been reported from UK estuaries has demonstrated tissue burdens in *Hinia reticulata* to be quantitative indicators of exposure concentrations in each phase. In all instances, uptake appeared to be a logarithmic function of time, with rapid initial uptake gradually decreasing, but not apparently achieving equilibrium, which may be explained by the factors just discussed. At all exposure concentrations, except very low levels (<3 ng l^{-1} TBT in seawater and ~67 ng g^{-1} in sediments) *Hinia reticulata* exposed to sediments accumulated significantly higher body burdens than those exposed to water alone. Furthermore, it was apparent that the bioconcentration factor for sediment-derived TBT increased as the sediment concentration increased, implying that the sediments become an increasingly significant source of TBT to *Hinia reticulata* as the degree of sediment contamination increases. An estimated 31 – 50% of the body burden appeared to be sediment-derived at low levels, but increased to 51 – 87% in more contaminated sediments. Conversely, it may be said that dissolved TBT is a more significant vector for bioaccumulation at very low environmental TBT levels.

Depuration and Metabolism

After toxicants enter an organism they may be stored or eliminated, and the kinetics of these processes are affected by the compound's metabolism, or lack of it. Many toxic compounds like TBT are hydrophobic and accumulate in the lipid rich compartments of tissues and cells.

Elimination of such compounds is facilitated by their transformation into water-soluble polar compounds. Such metabolism of a compound generally reduces persistence and toxicity, and increases elimination. TBT is believed to enter organisms through lipid membranes, and may be metabolised in two stages: Stage 1 involves the cytochrome P-450 dependent monooxygenase system (MFO) - a multicomponent enzyme system which is associated with the endoplasmic reticulum of the cell, - which hydroxylates TBT to a series of hydroxydibutyltin derivatives (Fish et al., 1976). The stage 2 reactions conjugate these compounds with sugars or sulphate, and these highly polar, water-soluble conjugates are rapidly eliminated from the organism.

There have been many studies on these enzyme systems in fish and crustaceans, where the system appears very active, but molluscs appear to have a lower cytochrome P-450 content and mixed function oxygenase system which results in TBT accumulation and slow depuration (Lee, 1996). It has been suggested that many of the TBT effects which have been observed in molluscs are related to this slow metabolism of TBT, binding of TBT metabolites to cellular proteins, and even the inhibition of the detoxifying enzyme systems by TBT (Langston et al., 1997; Lee, 1996).

Fish exposed to TBT in water exhibited TBT, DBT and MBT in their tissues (Ward et al., 1981) and a high level of MFO activity. TBT metabolites (primarily DBT) were predominantly found in the liver, indicating the importance of this tissue in TBT metabolism. Bioconcentration factors of 400 - 500 were reported for fish exposed to TBT in water, and when transferred to TBT-free water, the depuration half-life was found to be approximately 7 days (Ward et al., 1981).

Several marine invertebrate species such as the polychaete *Nereis virens* have also been shown to contain significant concentrations of DBT and MBT suggesting that they are able to metabolise TBT (Maguire and Tkacz, 1985). Decapod Crustacea have also been well studied after exposure to TBT in water and food, with various metabolites, primarily DBT, occurring in the hepatopancreas (Lee, 1985; Lee, 1986; Rice et al., 1989). The hepatopancreas of aquatic arthropods is important in the accumulation and metabolism of a range of toxicants and is a major site of MFO activity. In contrast to Crustacea, most molluscs show more limited ability to metabolise TBT. *Mya arenaria* exposed to TBT in water showed very little DBT in it tissues,

suggesting very limited ability to metabolise TBT (Langston et al., 1987). *Mytilus edulis* and *Crassostrea virginica* accumulated primarily TBT in the digestive gland and gills after exposure to TBT in food or water (Laughlin et al., 1986a; Lee, 1986). Oysters and other bivalves have very low MFO activity (Livingstone and Farrar, 1985) and as a result, may accumulate TBT to a greater extent than crustacea or fish, and therefore exhibit higher bioconcentration factors, and slower depuration rates (Table 4.8).

Table 4.8 Bioconcentration factors (from water) and depuration rates for TBT in some bivalve species.

Species	BCF	Depuration half-life	Reference
Crassostrea gigas	2000 - 6000	23 d	(Waldock et al., 1983)
Ostrea edulis	1000 – 1500		(Waldock and Thain, 1983)
Scrobicularia plana	8200 - 11600		(Langston et al., 1987)
Mya arenaria	122100 - 143900		(Langston et al., 1987)
Mytilus edulis	5000 – 70,000	14 – 56 d	(Laughlin, 1986; Salazar and Salazar, 1988; Bryan, 1989)

Marine gastropods have also been shown to be capable of metabolising TBT to DBT and MBT (Bryan et al., 1987; Bryan et al., 1989) but the rates were slow in comparison to crustaceans and fish, with half lives in the range 48 – 120 days.

The extraction processes used in this series of experiments on *Hinia reticulata* have allowed the speciation of butyltins in tissues to be estimated, and have revealed some interesting comparative trends.

During TBT uptake by *Hinia reticulata* at low, medium and high exposure concentrations, there was a significant reduction in the 'TBT' fraction (NaOH-washed hexane extract) as a percentage of the 'total organotin' fraction (Optisolv extract) indicating some metabolism of TBT, most likely via the MFO system as discussed. An exception occurred at high exposure levels where no significant reduction in the proportion of 'TBT' was apparent, suggesting that the metabolic process may have been inhibited at high concentrations of TBT.

Depuration experiments revealed further trends in TBT metabolism. Unfortunately, when depuration followed natural uptake, the initial variability in tissue burdens between individuals was high, so that it was difficult to determine depuration profiles and rates with accuracy. Nevertheless, the results showed that concentrations of butyltins in all extracts decreased with time with half-lives in the range 40 – 50 days. Injection of a fixed dose of TBT into *Hinia reticulata* prior to depuration avoided the level of uncertainty following natural uptake, and allowed better estimates of depuration and butyltin speciation changes to be made. This showed that depuration followed an exponential path with half-lives of ~26 days for 'total' butyltins and ~18 days for TBT. These rates are similar to those reported for other molluscs (Table 4.8), but are faster than the depuration rates (65 – 78 days) reported for *Hinia reticulata* collected from contaminated field sites, and allowed to depurate under laboratory conditions for 18 months (Stroben et al., 1992a). Depuration rates were also determined in *Hinia reticulata* and *Nucella lapillus* where the dose (2500 ng TBT) was injected, rather than taken up naturally, in an experiment similar to that described earlier, and showed half-lives for depuration to be 19.1 and 32 days respectively for these species (Stroben et al., 1992a). Therefore the depuration rates determined in the series of experiments described here appear to be in accordance with previous work on *Hinia reticulata*, and other molluscs, and demonstrate that the species is capable of TBT metabolism and excretion of the metabolic products.

Comparison of results for sediment-exposed and water-exposed individuals showed there to be significant differences in the proportion of TBT in each group, at all exposure levels. Sediment-exposed animals contained an average of 69.1% TBT while water-exposed individuals contained slightly less at 61.8%. Analyses of sediment and water extracts showed that in all experiments the proportion of TBT in sediments was much higher than in the overlying water, indicating that some degradation of TBT occurred within the sediment/water system. The higher TBT burden in sediment-exposed *Hinia reticulata* therefore probably reflects the higher proportion of TBT in sediments, but may also be suggestive of different uptake routes or mechanisms in animals exposed to the different environmental phases. Experiments described in the next chapter have attempted to gain further insight into the importance of these uptake pathways.

5 DISTRIBUTION OF BUTYLTINS IN THE TISSUES OF *HINIA RETICULATA*: LINKS WITH BIOACCUMULATION ROUTES AND METABOLISM

5.1 Introduction

The investigations described in this chapter were designed to extend those already presented, and attempt to reveal further, the specific routes whereby *Hinia reticulata* accumulates TBT from sediments and water, and where the compound is stored and metabolised in the tissues.

The design and functional biology of gastropod molluscs have been extensively described (Fretter and Graham, 1962; Graham, 1971; Hughes, 1986) but some of the more important features are reviewed here.

5.1.1 Gastropod form and function

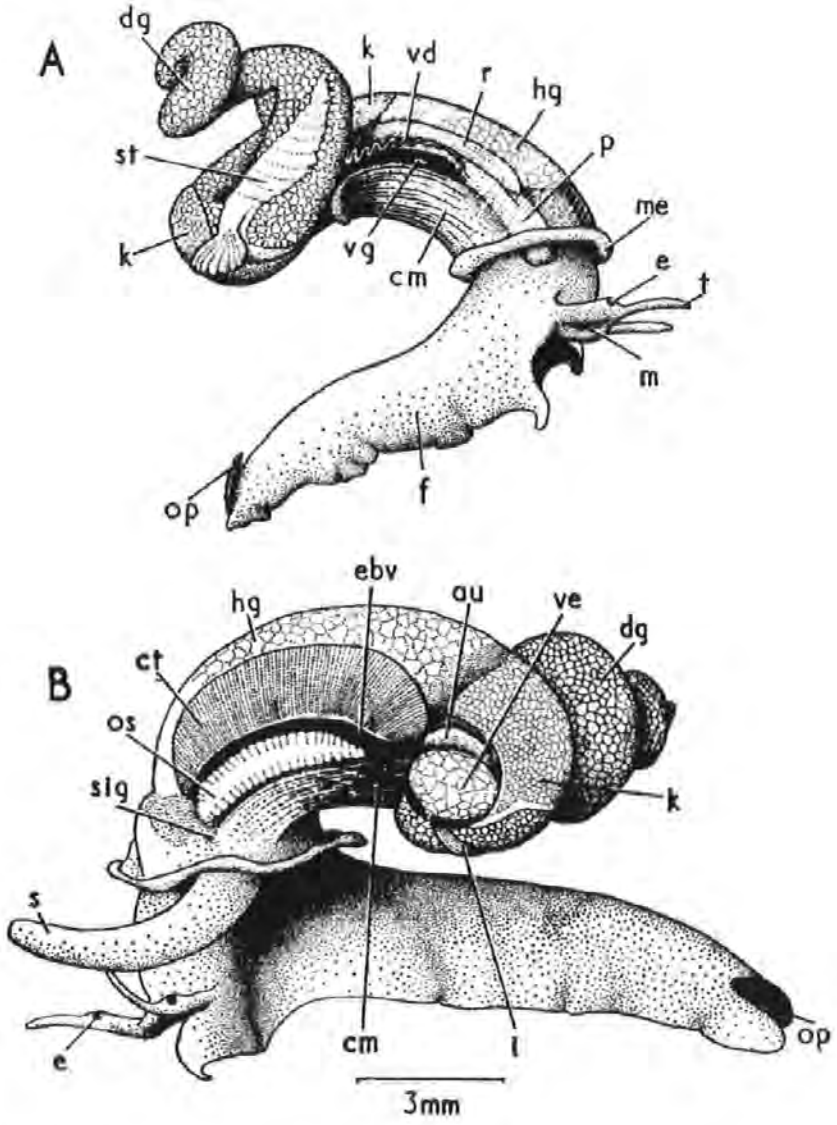
Coiling of the gastropod visceral mass has tended to suppress sets of organs on the right-hand side of the body, and in meso- and neogastropods these have been lost altogether (Figure 5.1). The heart has only one auricle (monotocardian) and the gill has filaments only on one side of its central axis (monopectinate). Neogastropods are distinguished by the radula, which never has more than three teeth per row, and by collection of glandular tissue in the oesophagus into a separate gland (Leiblein's gland) which is ducted to the oesophagus. The stomach is reduced to a simple sac, and lacks complex ciliary sorting areas. Neogastropods also have a well-developed proboscis, and an elaborate olfactory organ (the osphradium), together with a siphonal canal in the anterior lip of the shell to accommodate the siphon – a snout-like fold of the mantle, which directs inhalant water on to the osphradium. Soft tissues are attached to the central columella of the shell by the columellar muscle, which may be used either to push the animal out of the shell, or withdraw the body entirely within it, sealed with the horny operculum.

The body of the animal consists of a large muscular foot, mounted on top of which are the head and visceral mass. When moving over a substratum a layer of mucus is secreted from the sole of the foot and affords a temporary grip for gliding by the movement of cilia covering the sole, or by waves of muscular contraction.

Figure 5.1 *Hinia reticulata*: View of the whole animal removed from the shell

A. Viewed from the right; B. Viewed from the left

(Reproduced from (Fretter and Graham, 1962))



au, auricle; **cm**, columellar muscle; **ct**, ctenidium; **dg**, digestive gland; **e**, eye; **ebv**, efferent branchial vessel; **f**, foot; **hg**, hypobranchial gland; **l**, intestine; **k**, kidney; **m**, mouth; **me**, mantle edge; **op**, operculum; **os**, osphradium; **p**, penis within mantle cavity; **r**, rectum; **s**, siphon; **sig**, siphonal ganglion; **st**, stomach; **t**, tentacle; **vd**, vas deferens; **ve**, ventricle; **vg**, visceral ganglion

The head bears a pair of tentacles, each with an eye, and a retractable proboscis terminating in the mouth. The mouth opens into the buccal cavity with its cartilaginous odontophore over which the radula extends and which can be protruded slightly out of the mouth in the process of food gathering. The head merges into the visceral mass containing the stomach, digestive gland, intestine, kidney, heart and gonad. Food is digested extracellularly in the stomach by enzymes secreted by the digestive gland, although fine particles of partially digested food may be taken up by the digestive gland cells within which the final stages of digestion are completed. The relative importance of these extracellular and intracellular digestion processes differs between gastropod species (Hughes, 1986).

Metabolites dissolved in the blood (haemolymph) are circulated around the body in a system of arteries, veins and sinuses. Muscular movements during locomotion contribute to the circulation, but the haemolymph is also pumped by the heart, to the gill and kidney. In some gastropods the heart produces primary urine (pericardial fluid) by ultrafiltration across its thin wall. This passes directly to the kidney where it receives the nitrogenous products of metabolism prior to excretion.

In most prosobranchs, oxygen is carried in the plasma of the haemolymph, bound to the copper-associated pigment haemocyanin, which although it functions less efficiently than haemoglobin, saturates at lower oxygen tensions and allows some gastropods to survive poor aeration. Gaseous exchange occurs over exposed surfaces of the body and across the gill, their relative importance depending on the size and anatomy of the gastropod. The single gill of neogastropods projects into the mantle cavity and lateral cilia on the gill filaments drive the respiratory current in the opposite direction to the flow of blood to effect a counter-current system for gaseous diffusion. In most gastropods this current generated by the gill enters the mantle cavity to the left of the head and leaves on the right. Next to the gill lies the osphradium, a pleated chemosensory strip of specialised mantle tissue, which detects odours in the inhalant current. Among gastropods such as *Hinia reticulata* the osphradium superficially resembles a gill, and has a greatly increased surface area. A fold of the anterior pallial margin forms a fleshy siphon which directs the inhalant current over the osphradium, and which can be extended into a long flexible hose suitable for intimate exploration of olfactory stimuli.

The rectum discharges faeces to the right of the head in the exhalent current thereby minimising the risk of fouling the mantle cavity. With its central role in respiration, chemoreception and waste disposal the mantle cavity must be kept clear of detrital material. The hypobranchial gland, a strip of mucus-secreting epithelium, runs parallel to the gill and osphradium, and detrital particles entering on the inhalant current adhere to the mucous secretions and are transported across the mantle cavity by ciliary action to the exhalent region where they are removed as pseudofaeces.

5.2 Materials and Methods

The experiment described in this chapter is a repeat of a previous experiment not reported in this thesis. The reason for this is that in the initial experiment, TBT exposure concentrations in sediments and water were too low to obtain reliable measurements of ^{14}C-TBT in some of the very low mass tissues, particularly the kidney. Hence the exposures used in this experiment are very high by environmental standards, but I feel they are justified within the aims and context of this experiment.

The standard experimental system was employed with an upper tank containing sediment and a lower tank receiving water runoff from the upper one. Sediment in the upper tank was collected from St. Johns Lake (Figure 3.19) and spiked at approximately 10 μg g^{-1} TBT. Analysis of the spiked sediment showed that the initial concentration of hexane extractable butyltin (TBT+DBT) was 10.2 μg g^{-1}, while a repeat analysis some 3 months later showed this to have decreased to 8.7 μg g^{-1} (TBT+DBT). After the initial spiking of the sediment, by the process described in chapter 2, The experimental system was left to equilibrate, prior to the addition of *Hinia reticulata*. Butyltin concentrations in both tanks were monitored regularly throughout this period, and during and after the addition and removal of *Hinia reticulata*.

A feature worthy of mention, since it impacts upon the results obtained, was that during the equilibration period the peristaltic pump supplying seawater to the system developed a fault, and had to be sent away for repair, leaving the system essentially static, with only the air supply maintaining a residual circulation.

Once the system had equilibrated and settled down (some 5 weeks after the sediments had been spiked) 120 – 130 *Hinia reticulata* were added to each experimental tank. The reason for

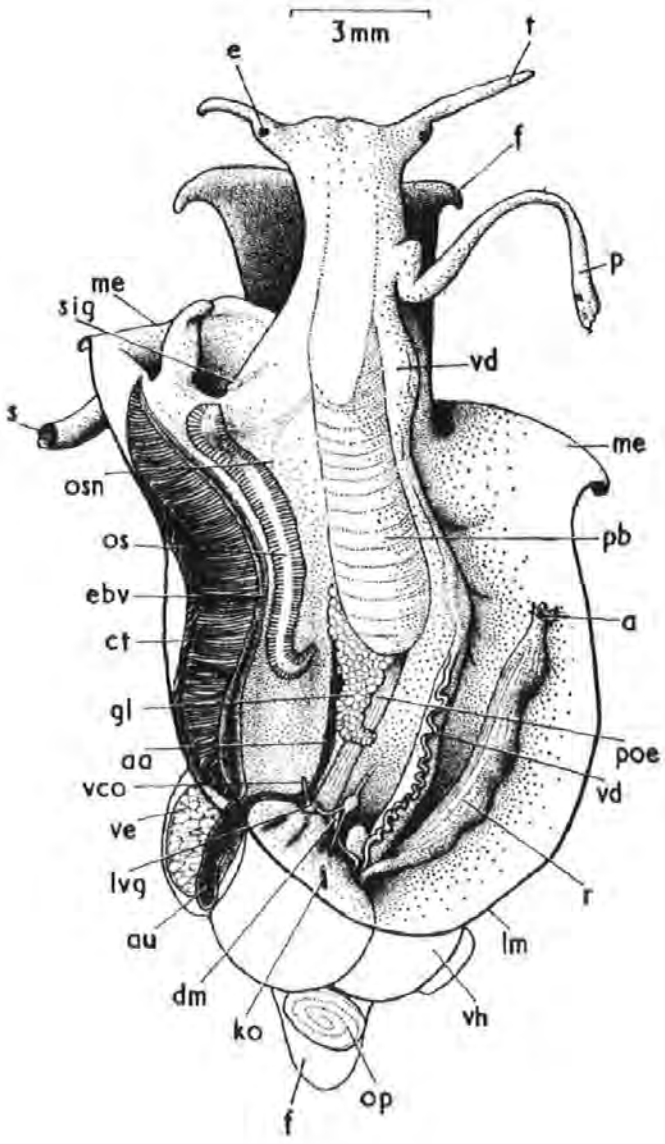
such large numbers of individuals will be apparent later in this section, but an effect of this was that bioturbation was much greater than in previous experiments, and resulted in significantly different butyltin concentrations in the waters of the two tanks while *Hinia reticulata* were resident. Hence both tanks were monitored separately.

Ten *Hinia reticulata* were removed for analysis from each tank after 5, 12, 19, 26, 33 and 40 days exposure. At the end of the exposure period all remaining animals in each tank were removed, and caged in flowing seawater for a further 50 days depuration. Ten of these animals from each exposure regime were removed on days 47, 54, 61, 75 and 89 after their introduction to the experiment.

At every sampling interval each batch of 10 individuals were measured and the whole soft tissues very carefully removed by cracking the shell in a vice and separating the columellar muscle from its attachment. The live, shell-less animals were then narcotised in magnesium chloride solution (75 g l^{-1}) and dissected under a low power microscope. A longitudinal cut along the mid-dorsal line of the mantle revealed much of the internal structure (Figure 5.2) although this could sometimes be obscured by a dark surficial pigmentation in some animals. However, it was found that by gently brushing the tissues with a soft artist's paintbrush this surface coating could easily be removed. Once individual tissues could be identified, the gill and osphradium were carefully dissected away, still attached to a strip of mantle tissue. Next, the kidney at the anterior end of the visceral mass was removed, followed by the digestive gland and empty stomach (animals were not fed during the experiment). Finally, the large muscular head/foot tissue was separated from the remaining tissues originally contained within the shell, largely comprising the hypobranchial gland and withdrawn proboscis. Due to the very low mass of some of these tissues, particularly the gill/osphradium and the kidney, the tissues from all ten individuals in each exposure regime were pooled into pre-weighed vials, re-weighed and homogenised prior to analysis. This had the unfortunate effect that it did not allow statistical analyses to be made on the results, but it was unfeasible to analyse individual tissues from each animal with sufficient reliability for meaningful comparisons to be made.

Figure 5.2 Diagram of *Hinia reticulata*: to show the animal removed from the shell with the mantle skirt cut longitudinally along the mid-dorsal line to display internal structure.

(Reproduced from (Fretter and Graham, 1962))



a, anus; **aa**, anterior aorta; **au**, auricle; **ct**, ctenidium; **dm**, diverticulum of male duct opening to mantle cavity; **e**, eye; **ebv**, efferent branchial vessel; **f**, foot; **gl**, gland of Leiblein; **ko**, opening of kidney to mantle cavity; **lm**, cut edge of mantle skirt; **lvg**, left visceral ganglion; **me**, mantle edge; **op**, operculum; **os**, osphradium; **osn**, osphradial nerve; **p**, penis; **pb**, proboscis; **poe**, posterior oesophagus; **r**, rectum; **s**, siphon; **sig**, siphonal ganglion; **t**, tentacle; **vco**, left part of visceral loop; **vd**, vas deferens; **ve**, ventricle; **vh**, visceral hump.

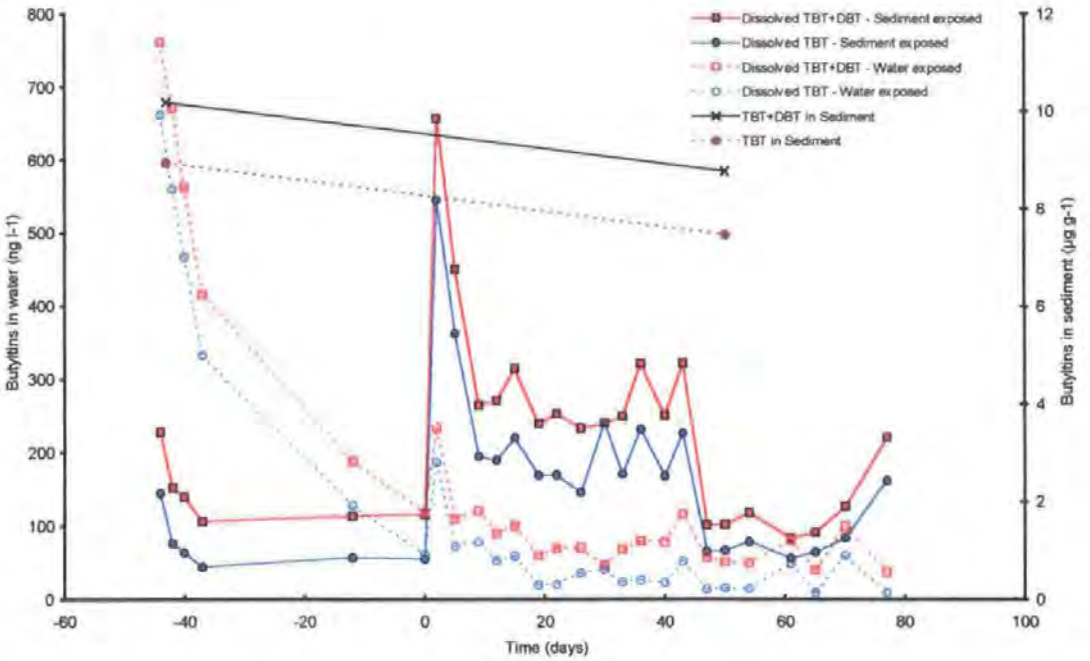
5.3 Results

5.3.1 Water and sediments

Butyltin concentrations in sediments, and in the water of both experimental tanks (Figure 5.3) show some interesting features. High initial concentrations of butyltins in the water-only exposure system were believed to be the result of two factors. Firstly, just after the sediments were spiked and added to the experimental system the overlying water in the sediment exposure tank was very high due to desorption. This would have carried over to the water-only tank as the result of clean seawater being pumped into the sediment-exposure tank. At that point the pump began to fail, resulting in very reduced water exchange which was not noticed for several days. However, once the pump was repaired and normal flows restored, butyltin concentrations in the waters of both tanks approached similarity until they finally equalised and *Hinia reticulata* were added and the uptake experiment was initiated. The sudden addition of approximately 120 – 130 animals to the tank containing spiked sediment (Day 0 in Figure 5.3) resulted in sudden and dramatic bioturbation of the system, as seen before in previous experiments, but now on an extreme scale due to the number of whelks involved. The result was a sharp increase in the TBT concentration in the overlying water in the sediment tank, together with some carry-over to the water-only tank (Figure 5.3). During the whole exposure period (up to day 40 in Figure 5.3) TBT average concentrations in the sediment tank (220 ng l^{-1}) remained significantly higher (P=0.000017, Student's t-test) than those in the water-only exposure regime (54 ng l^{-1}), presumably as a continued result of bioturbation, although after a few days the animals appeared to settle into their new environment and moved around much less than when initially introduced. After the point at which all *Hinia reticulata* were removed (on day 40 of the exposure period) the difference in TBT concentrations in both tanks reduced considerably, though those in the sediment tank (100 ng l^{-1}) still remained significantly higher (P=0.01, Student's t-test) than those in the water-only system (28 ng l^{-1}).

The initial concentration of hexane extractable butyltins (TBT+DBT) in the sediment was 10.2 µg g^{-1}, but had decreased to 8.7 µg g^{-1} (TBT+DBT) some 3 months later, demonstrating the release of TBT from sediments to the overlying water in addition to uptake by *Hinia reticulata*.

Figure 5.3 Butyltin concentrations in sediments and water during exposure of *Hinia reticulata*

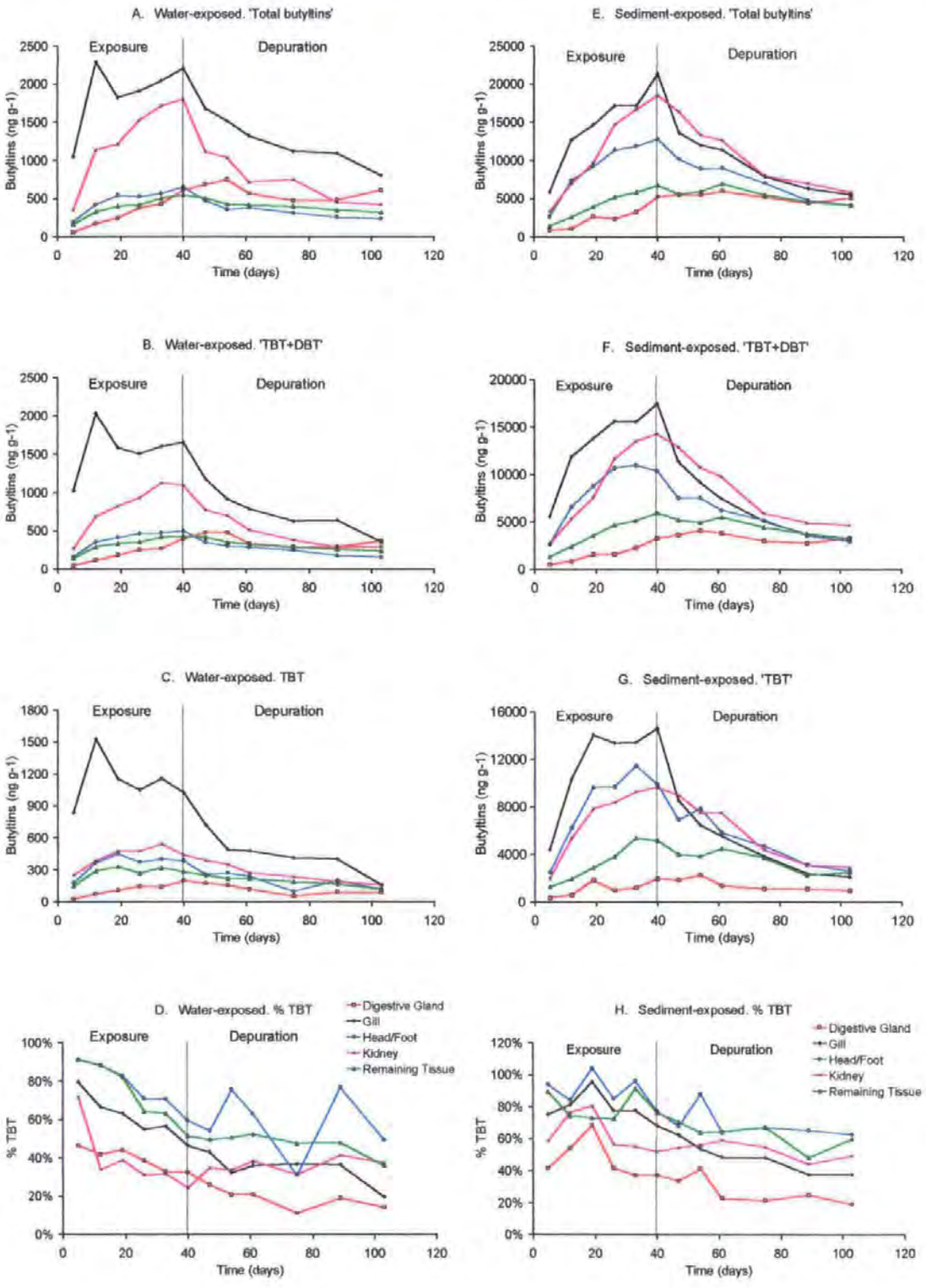


5.3.2 *Hinia reticulata*

Results for butyltin concentrations in the tissues of *Hinia reticulata* during uptake and depuration are presented in Appendix 6. These results are more revealing when presented graphically (Figure 5.4) and show some interesting differences between sediment-exposed and water-only exposed animals. Despite the fact that TBT concentrations in the overlying waters of the sediment tank were about 4 times higher than those in the water-only tank, the concentration of total butyltins in sediment-exposed *Hinia reticulata* were approximately 10 times higher than in animals exposed only to water. This re-affirms that additional TBT uptake occurs when *Hinia reticulata* is exposed to TBT contaminated sediment in addition to TBT laden water.

Comparison of the patterns of accumulation within the different tissues reveals that in *Hinia reticulata* which were exposed to TBT in the water, the highest concentrations of total butyltins occurred in the gill/osphradium, and in the kidney (Figure 5.4A). The head/foot, digestive gland and remaining tissues all exhibit much lower concentrations. A very similar pattern was seen for the concentrations of hexane extractable butyltins (approximating to TBT+DBT) with the

Figure 5.4 Uptake and depuration profiles for butyltins in selected tissues of water-exposed and sediment-exposed *Hinia reticulata*



highest concentrations again in the gill/osphradium (Figure 5.4B). Concentrations in the kidney however, appear to be lower in comparison to the gill/osphradium than was seen for 'total butyltins'. If the concentrations derived from NaOH washed hexane extracts (approximating TBT) are considered (Figure 5.4C) it can be seen that concentrations in the kidney were very much lower in comparison to the gill/osphradium. Viewed together, these patterns for the accumulation of the different butyltin species suggest that uptake from seawater occurs primarily across the respiratory surfaces, although it is possible that TBT may be taken up across other exposed surfaces as well. The decrease in concentrations through the series Total>TBT+DBT>TBT in the kidney provides some evidence that butyltins accumulated in the body tissues have been metabolised through a debutylation sequence and excreted via this organ.

The lowest percentages of TBT compared to total butyltins were seen in the tissues of the digestive gland (Figure 5.4D) and it is almost certain that this is the major site of TBT metabolism since this tissue is known to contain the MFO system in many gastropods.

A very similar distribution pattern for butyltins was seen in *Hinia reticulata* which had been additionally exposed to TBT contaminated sediments (Figures 5.4E-H). There was however, one very noticeable difference: Besides exhibiting high total butyltin concentrations in the gill/osphradium and kidney tissues, the concentrations in the head/foot of sediment-exposed *Hinia reticulata* was very much higher, relative to the other tissues, than it was in animals exposed solely to TBT in seawater (Figure 5.4E). This suggests that TBT uptake additionally occurs across the surface of the foot when *Hinia reticulata* is in direct contact with TBT-contaminated sediment.

These results presented so far, have considered only the *concentrations* of butyltin species in the different tissues, and do not reflect the actual burdens contained in each tissue type. If the mean dry weights of each tissue are calculated for the animals sampled at each interval, it is then possible to estimate the burdens in each tissue type. These results are presented in Appendix 7, and show that, taking into account the masses of the different tissues, the highest accumulated total, and hexane-extractable (TBT+DBT) burdens occurred in the head/foot,

Figure 5.5 Butyltin burdens in tissues of water-exposed and sediment-exposed *Hinia reticulata*.

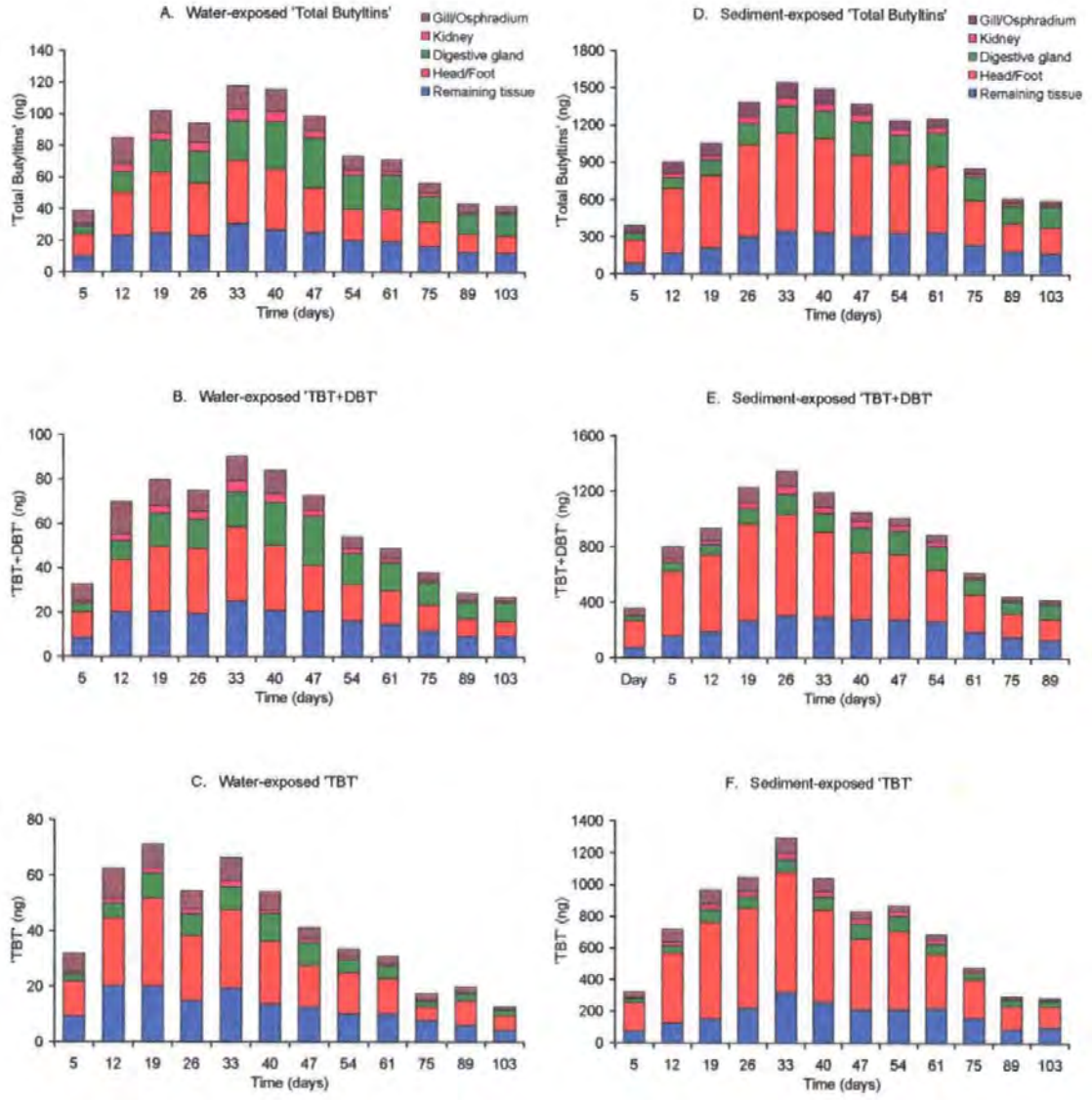
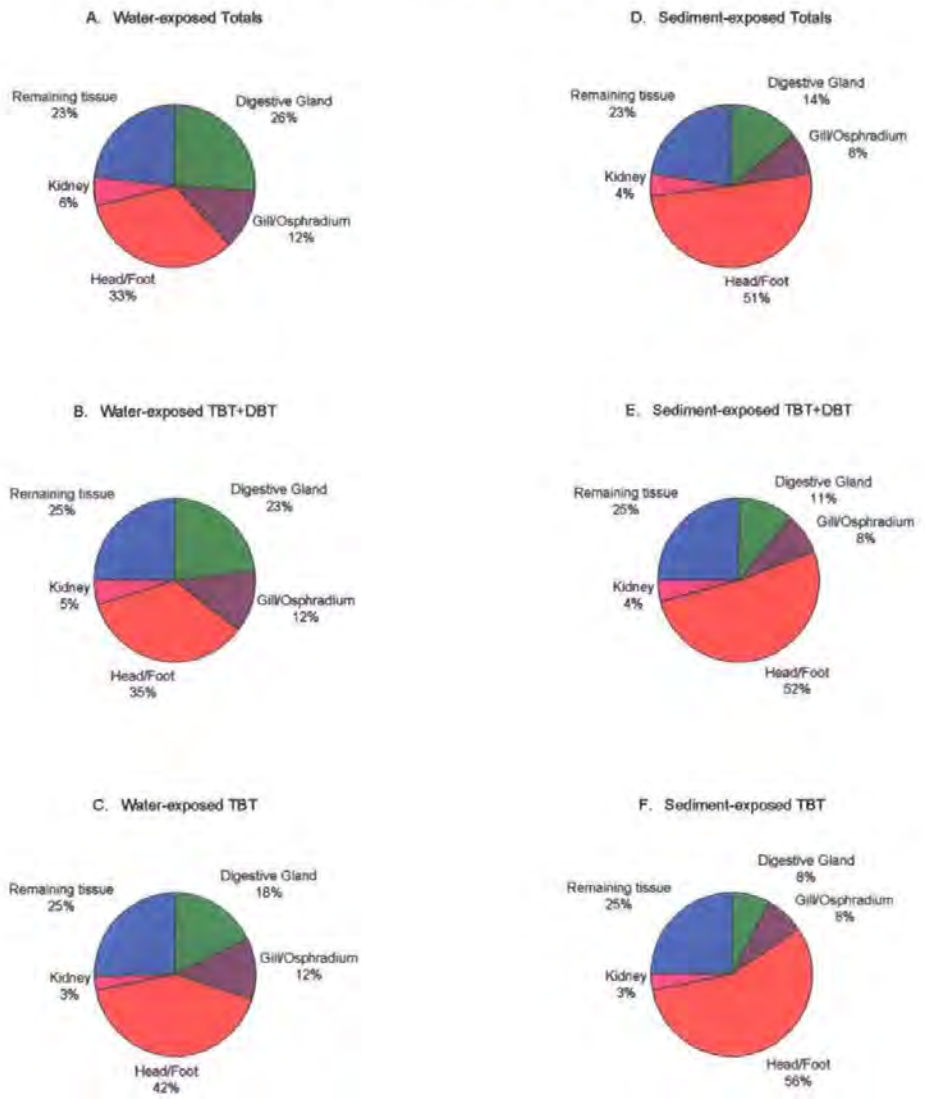


Figure 5.6 Distribution of butyltin burdens between the tissues of *Hinia reticulata* after 40 days exposure to TBT contaminated sediments and water.



under both exposure regimes, together with high burdens in the remaining body tissues (Figure 5.5). In sediment-exposed animals however, the total burden in the head/foot appeared to be proportionally higher than in water-only exposed animals. Figure 5.6 summarises the distribution of butyltin burdens in *Hinia reticulata* at the end of the exposure period (day 40) under both exposure regimes. This shows that water-exposed *Hinia reticulata* accumulated 33 – 42% of the body burden in the head/foot, while sediment-exposed animals contained slightly more, at 51 – 56%. The proportion of the burden in the remaining tissues (largely comprising the hypobranchial gland and withdrawn proboscis) was very similar across both exposure regimes at 23 – 25%. Gill/osphradium tissues in water-exposed animals contained 12% of the

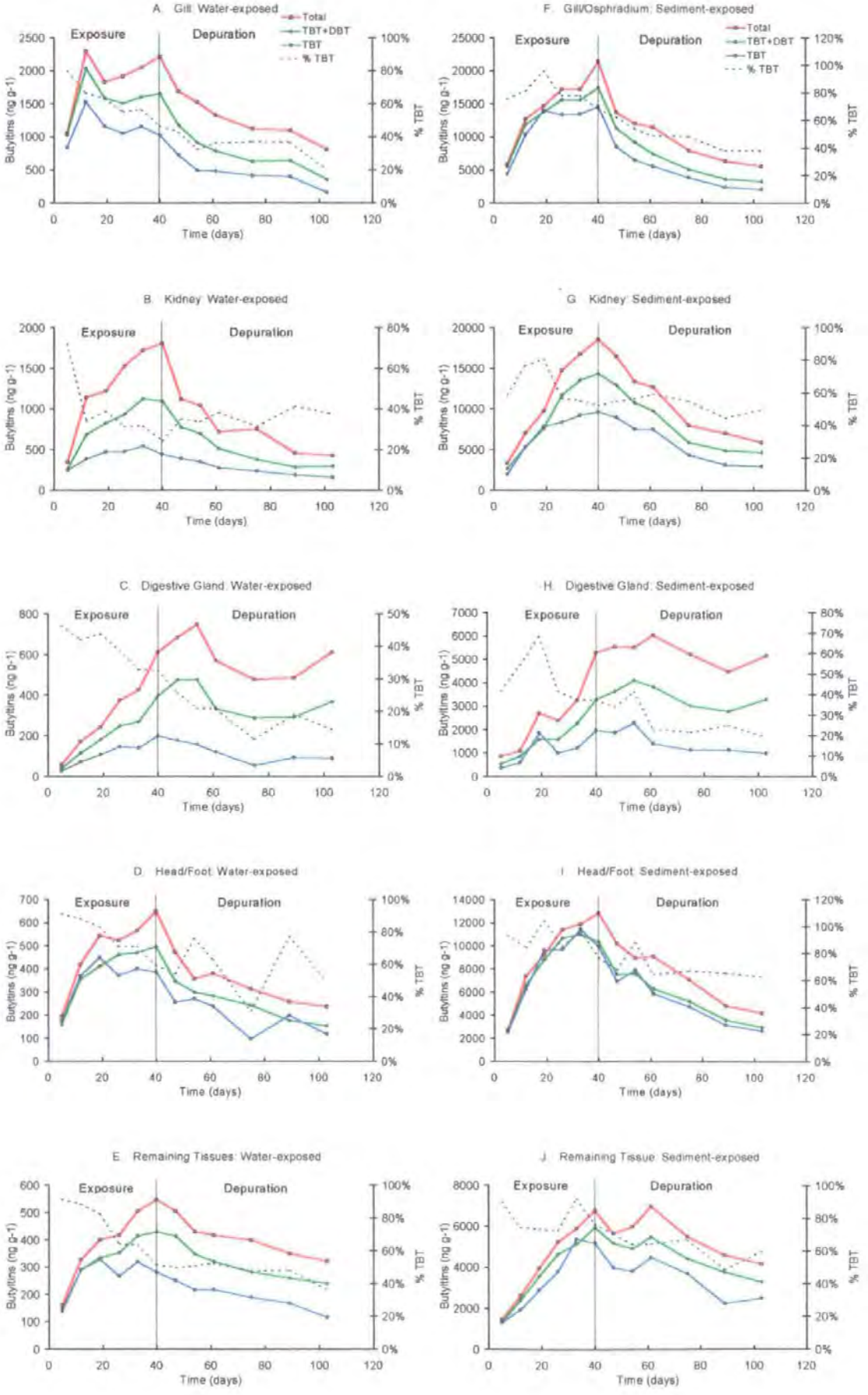
body burden, but this was slightly lower, at 8% in *Hinia reticulata* which were additionally exposed to TBT contaminated sediment. Burdens in the digestive gland represented 18 – 23% of the total in water-exposed animals, but only 8 – 14% in those exposed to sediment. Notably, the proportions in the digestive gland decreased through the series Total>TBT+DBT>TBT. This was the only tissue to exhibit such a reduction and provides further evidence for the debutylisation of TBT in the digestive gland of *Hinia reticulata*.

More information on the metabolism of TBT in *Hinia reticulata* is revealed by viewing the data in Appendix 6 in a different arrangement, comparing the concentrations of the different butyltin species in each tissue (Figure 5.7). For sediment-exposed *Hinia reticulata* there appears to be very little degradation of TBT in the gill/osphradium, head/foot or the residual tissues during the exposure period, although in all cases the percentage TBT decreased during depuration. However, the percentage of the body burden contained in the digestive gland decreased through the series of extracts 'Total'>'TBT+DBT'>'TBT' during exposure and depuration, during which time the percentage TBT was reduced to 20-30% of the total (Optisolv extractable) concentrations.

This pattern suggests that *Hinia reticulata* exposed to TBT in sediments (where the proportion of TBT was high) accumulate TBT predominantly across the respiratory surfaces and the exposed tissue of the head/foot, which is in contact with the sediment and pore water as the animal burrows. These butyltins may subsequently be transported via the haemolymph to other body tissues where they accumulated or metabolised. The results presented here suggests that metabolism of TBT predominantly occurs in the digestive gland of *Hinia reticulata*. Once metabolised the debutylated products, which are more polar in nature than TBT, may be excreted via the kidney.

An almost identical pattern for butyltin species was seen in the tissues of *Hinia reticulata* which had only been exposed to TBT in seawater, although in this case the percentage of TBT in the gill/osphradium, head/foot, and the remaining tissues was considerably lower than in sediment-exposed animals, which probably reflects the lower proportion of TBT in seawater seen throughout the experiments.

Figure 5.7 Butyltin speciation in individual tissues of *Hinia reticulata* during uptake and depuration of TBT in sediments and water



5.4 Discussion

The results presented in this chapter provide further evidence of the importance of different uptake routes for TBT in *Hinia reticulata* exposed to TBT in sediments and water. Analyses of gill/osphradium; kidney; digestive gland/stomach/gonad; head/foot and the residual tissues shows that the highest concentrations of all butyltin extracts occurred in the gill/osphradium under both exposure regimes. This rapid increase in concentrations in the gill/osphradium tissues during uptake, together with rapid decrease during depuration strongly suggests that uptake across the respiratory surfaces is an important route for TBT accumulation in *Hinia reticulata*. In sediment-exposed *Hinia reticulata*, most of the 'total butyltin' burden in the gill was present as TBT at all times, suggesting little metabolism of TBT in this tissue. Water-only exposed animals however, showed a lower proportion of TBT in the gill/osphradium which, given the lower percentage of TBT in the overlying water compared with the sediment, could indicate some uptake of DBT across the gill membrane. However, there is evidence that in *Nucella lapillus* most tissue DBT originates from TBT degradation in the body rather than uptake from seawater (Bryan et al., 1988). High levels of TBT in gill tissues have also been reported in fish, *Leiostomus canthurus* (Lee, 1986) and blue crabs *Callinectes sapidus* (Lee, 1985) and would be expected for aquatic organisms exposed to lipophilic compounds.

A very interesting feature of the results presented here for *Hinia reticulata* is that when additionally exposed to TBT contaminated sediments, the concentration of all butyltin extracts in the head/foot tissue increased considerably, relative to the other tissues, compared with animals which had been exposed only to TBT in seawater. This suggests that uptake across the surface of the foot – a large, flat area of tissue – is an important additional route for TBT accumulation in sediment-exposed *Hinia reticulata*. If the mass of this tissue is taken into account besides the butyltin concentrations it is apparent that the head/foot forms the largest single reservoir of butyltins in *Hinia reticulata*, containing over 50% of the body burden in sediment-exposed animals. Water-exposed animals accumulated less, at 33 – 42% of their body burdens in the head/foot, indicating that it is an important 'sink' for butyltins in *Hinia reticulata* under all exposure regimes. As in the gill/osphradium tissue, very little degradation of TBT was apparent in the head/foot indicating that it is probably not a major site for TBT metabolism. In many gastropods such as *Patella, Thais* and *Neritina* the pedal muscle fibres

are tightly packed in the foot, with few haemocoelic spaces. In contrast, the foot of some nassariid species e.g. *Bullia* contains less tightly packed muscle fibres and a large blood-filled sinus. The foot of *Nassarius kraussianus* has been shown to be somewhat intermediate (Trueman and Hodgson, 1990), with moderately dense muscle fibres and blood vesicles. Therefore, if the structure of the foot in *Hinia reticulata* is similarly composed, and well supplied with blood, it is perhaps not surprising that it is capable of acting both as a reservoir, and an uptake surface. There have been several previous reports of the uptake of metals across the foot of gastropods (Ireland, 1982; Ireland, 1983) while slug skin is believed to be enzymatically equipped to absorb water and ions, and structurally, closely resembles absorptive epithelium (Newell, 1977). What is not clear from the present study, and which in practice may be difficult to determine, is whether the uptake vector is the pore water, or direct partitioning of TBT from the sediment to the tissue, or indeed a combination of the two. The accumulation of TBT in tissues of *Hinia reticulata* and *Ocenebra erinacea* collected from the coasts of Brittany and Normandy (Ohelmann et al., 1992; Stroben et al., 1992b) showed 22 – 26% of the body burden to be present in the foot, though the precise details of dissection were not given and may not therefore be comparable to the head/foot tissues described here. Clearly, the uptake of sediment-bound contaminants across the head/foot of *Hinia reticulata* could be an area for further research.

In this study, after the gill/osphradium, the next highest concentrations of 'total' and hexane extractable (TBT+DBT) butyltins were seen in the kidney of *Hinia reticulata*, although the concentration of TBT was lower indicating that this excretory organ was probably removing TBT and its metabolic products from the haemolymph, besides the normal products of nitrogenous metabolism. High concentrations of TBT have also been reported in the kidney of *Hinia reticulata* (Stroben et al., 1992b) and *Ocenebra erinacea* (Oehlmann et al., 1992) although neither of these authors report the concentrations of other butyltin species.

The residual tissues of *Hinia reticulata*, remaining after dissection and removal of other tissues largely comprised the hypobranchial gland and proboscis with some of the mantle tissue. This formed a remarkably constant reservoir of gradually accumulated butyltins under both exposure regimes and was generally second to the head/foot in terms of its accumulated burden. Once again there appeared to be little evidence of TBT degradation in these composited remains

suggesting little TBT degradation therein, although relatively lower TBT burdens in water-exposed animals were generally reflective of the lower proportions of TBT seen in all tissues of *Hinia reticulata* under this regime.

Far greater changes in butyltin speciation were seen in the tissues of the digestive gland/stomach/gonad complex of *Hinia reticulata* in this study. This tissue formed a reservoir of 18 – 26% of the body burden in water-exposed animals, and 8-14% in those additionally exposed to TBT in sediment, and in both cases the burdens decreased through the series 'Total > 'TBT+DBT' > 'TBT'. Higher proportions of the total body TBT burden (47.52%) have been reported for field-sampled *Hinia reticulata* (Stroben et al., 1992b) and *Ocenebra erinacea* (57.08%) (Oehlmann et al., 1992), although in both these cases, where animals were collected fresh from the natural environment, it is highly likely that they may have recently ingested TBT contaminated food. This has been shown to be an important route for TBT uptake in *Hinia reticulata* (Stroben et al., 1992a) and in *Nucella lapillus* (Bryan et al., 1989).

While butyltin concentrations in the digestive gland gradually increased during the exposure phase, they appeared to be one of the slowest tissues to depurate butyltins. This perhaps suggests that TBT, accumulated in the various reservoirs discussed, was gradually metabolised in the digestive gland, and excreted as the more polar metabolic products via the kidney.

The metabolism of TBT by aquatic organisms has now be widely investigated and reviewed (Lee, 1996). The metabolic process has been mainly studied in fish liver and the hepatopancreas of aquatic arthropods where the enzyme systems concerned are well developed. Metabolism is believed to occur in two phases. Phase one reactions involve the cytochrome P-450 dependent monooxygenase system (MFO). Briefly, the TBT binds to the oxidised cytochrome P-450 (Fe^{3+}) and the complex undergoes reduction to cytochrome P-450 (Fe^{2+}), which then interacts with oxygen. A hydroxylated substrate (such as hydroxybutyldibutyltin) and a molecule of water then leave the re-oxidised cytochrome P-450, which is in turn reduced by electrons from NADPH carried by NADPH cytochrome P-450 reductase. Superoxide anions (O_2^-) formed during the reaction may participate in the hydroxylation of the substrate. This process occurs in the endoplasmic reticulum of fish liver

cells or cells in the hepatopancreas tubule of crustaceans where it is believed that TBT and its metabolites are distributed between the outer cell membrane, cytoplasm and the different organelles, and may be 'dissolved' in lipid, or bound to proteins in the membranes and organelles (Lee, 1996). Phase two enzyme systems (such as glutathione S-transferase) then conjugate the hydroxybutyldibutyltin to sugars such as glucose, or glutathione, or sulphate. These highly polar compounds would then be rapidly eliminated from the animal.

This system is less well developed in molluscs, which generally have a low mixed function oxygenase (MFO) activity, primarily associated with the digestive gland. (Lee, 1996) hypothesised that many of the effects of TBT seen in molluscs may be linked to the enzymes involved in TBT metabolism but that the biochemical basis for the observed effects is not fully understood. Many hormones share common metabolic pathways with toxicants, which may give rise to interactions. For instance cytochrome P-450 systems control the conversion of cholesterol into a variety of hormones (e.g. testosterone) so that inhibition or stimulation of these systems could result in changes in hormonal production or clearance. Thus the binding and inhibition of cytochrome P-450 by TBT may result in the observed production of testosterone by female neogastropods (Spooner et al., 1991). In vertebrates, a calcium metabolism cytochrome P-450 system is involved in the synthesis of vitamin D, which in turn regulates calcium metabolism. Thus Lee (1996) speculates that abnormal shell growth in oysters after TBT exposure may be related to inactivation of this cytochrome P-450 system. Clearly these systems warrant further investigation.

6 BIOAVAILABILITY OF TBT FROM DIFFERENT SEDIMENT TYPES AND ITS ACCUMULATION BY *HINIA RETICULATA*

6.1 Introduction

There have been several investigations into the *effects* of sediment-bound TBT, although these are far outweighed by studies on the effects of dissolved TBT. A solid-phase toxicity bioassay with spat (2-3 mm in length) of the sediment dwelling bivalve *Scrobicularia plana*, using natural sediments showed that both growth and burying activity were significantly affected in TBT contaminated sediments, implying ecological effects in the field such as increased predation (Ruiz et al., 1994a).

Anomalies between the concentrations of Sn in field populations of *Scrobicularia plana* and the Sn concentrations in 1M-HCl extracts of their native sediments (taken as a surrogate for bioavailable metals) particularly in harbours and areas of high boating activity (Langston et al., 1990) were subsequently shown to be correlated to the TBT concentrations in these sediments (Langston and Burt, 1991). Laboratory studies revealed that for *Scrobicularia plana*, there was a predominantly particulate component to TBT accumulation, with ingestion of TBT-contaminated sediment being the major vector for accumulation in this deposit-feeding clam, although some TBT was accumulated from water when this was the sole route of exposure (Langston and Burt, 1991). Sediments containing 25 $\mu g\ g^{-1}$ TBT were found to be acutely toxic to *Scrobicularia plana*, while field surveys revealed *Scrobicularia plana* to have almost disappeared in areas of the Solent, UK, where sediment-TBT concentrations were >0.75 $\mu g\ g^{-1}$ (Langston and Burt, 1991).

Recent investigations (Meador et al., 1997) have focussed on the toxicity of sediment associated TBT to 3 species of infaunal invertebrates: the polychaete *Armandia brevis* (a non-selective deposit feeder); and the amphipods *Rhepoxynius abronius* (a meiofaunal predator) and *Eohaustorius washingtonianus* (a detritivore). In particular, sediment organic carbon and its effects on partitioning and bioaccumulation of TBT by these biota were studied. Artificially composited natural sediments were employed as substrates and organic contents were manipulated by the addition of natural organic detritus. By varying sediment organic carbon content and holding grainsize constant, a 4 – 5 fold increase in LC_{50} and tissue accumulation

was observed as the sediment organic levels were reduced. LD_{50} values determined in all 3 species were very similar, and significant mortality was observed when tissue concentrations reached 35 – 80 μg g^{-1} TBT. It was proposed from these results that organic carbon association of TBT in sediments strongly influences the bioaccumulation and toxicity of TBT through its regulation of interstitial water TBT concentrations, and that dissolved TBT in these pore waters accounted for almost all of the TBT found in tissues of the test organisms, such that their mode of feeding was not an important factor in the determination of TBT uptake.

Quantification of the bioavailability of sediment-sorbed chemicals requires all avenues of exposure to be considered. Organisms in the water column are primarily exposed to toxicants released by the sediment back into the overlying water. Benthic species however, may be additionally exposed to the toxicant in the sediment interstitial (pore) water, and directly, through the ingestion of contaminated sediment particles (Anderson et al., 1987) although the latter does not appear to be a significant vector in the case of adult *Hinia reticulata* employed in this research programme. Therefore assessment of the bioavailability of sediment-associated compounds to benthic organisms, will be inherently more complex than the availability of dissolved compounds to pelagic organisms, or those which solely inhabit rocky surfaces and which do not routinely come into contact with sediment substrates.

The sequestration of TBT by sediment particles and its subsequent sorption behaviour will therefore be of paramount importance in controlling the proportions of TBT in each phase in a sediment water system. Furthermore, the nature of this partitioning process will vary with different sediment types as demonstrated and discussed earlier in this thesis (Chapter 3).

Despite the evidence that sediment-associated TBT is entering and affecting biological systems, the processes responsible for its transfer from sediments to organisms, and the physicochemical and environmental factors modifying these processes, remain ill-defined, with little published data available on this subject for TBT. Non-polar organic contaminants have received greater interest, and the variables that influence the transfer of these compounds from sediments to biota have been more widely investigated. As a brief summary of the major points, the main approach has been to compare the organism contaminant concentration to the concentration in the sediment as an accumulation factor (AF). This is calculated as the ratio of

the contaminant in the organism normalised to the lipid content, divided by the concentration in the sediment normalised to the organic carbon content. This assumes that non-polar contaminants partition predominantly to sediment organic carbon and organism lipids, with interstitial water as the transfer medium. It also assumes the sediment to be the only source for bioaccumulation with no contribution from the overlying water.

Clearly, from the results presented in this thesis for the partitioning of TBT between natural waters and sediments, and the uptake of TBT from these phases by *Hinia reticulata*, such a model is not appropriate, although some aspects may be relevant to the overall discussion of sediment-TBT bioavailability.

The objective of the experiment described in this chapter was to measure the accumulation of TBT by *Hinia reticulata* exposed to different sediment types (selected from the range of sediments tested as part of the investigation into variation of K_d with sediment type) approximating those types with a low, medium and high adsorption capacity for TBT. By comparison of whole body burdens accumulated by *Hinia reticulata* during exposure to the sediments and overlying water, and to the overlying water alone, it was expected that significantly different TBT burdens would be accumulated from the different sediment types (The null hypothesis being that no significant difference occurred). Furthermore, by analysing individual tissues including the gill, kidney, digestive gland, head foot and remaining tissues it was hoped that some indication of the relative importance of different uptake routes in the different sediments and exposure regimes might be revealed.

6.2 Materials and Methods

Following earlier investigations on the variation of sediment partition coefficients (K_d) with different sediment types (reported in section 3.3.6) three different sediments were selected as substrates for this investigation. The sediments were: Talland Bay – a predominantly sandy coastal sediment with low K_d (248 l kg^{-1}); East Looe – a sandy estuarine silt with a moderate capacity for TBT (K_d ~8300 l kg^{-1}) and St. John's Lake – a fine estuarine silt with a high K_d (~16700 l kg^{-1}). Details of these sediments were presented in Table 3.13. One kilogram (wet weight) batches of each of the sediments were spiked with ^{14}C-TBT to approximately the same concentration (1000 ng g^{-1} TBT dry weight) using the protocol described (Section 2.2). These

sediments were then placed into each of three polycarbonate tanks in the standard *Hinia reticulata* exposure protocol (Section 4.2), each tank receiving a pumped seawater supply, and overflowing into lower tanks containing water only.

These systems were then allowed to equilibrate for one week, after which time 60 *Hinia reticulata* were added to each of the 6 tanks in the system. These animals were exposed for a period of up to 2 months. Water and sediment samples were collected regularly throughout the experimental period, and *Hinia reticulata* were sampled from each exposure regime after 10, 30 and 60 days exposure. At each of these sampling intervals 10 *Hinia reticulata* from each tank were analysed individually for their accumulated whole body TBT burdens, while a further 10 individuals were dissected (as described in the previous chapter) and the pooled gill/osphradium, kidney, digestive gland, head/foot and remaining tissues analysed to reveal any differences in TBT distributions between the tissues of animals exposed to different sediment types.

6.3 Results

6.3.1 Exposure concentrations

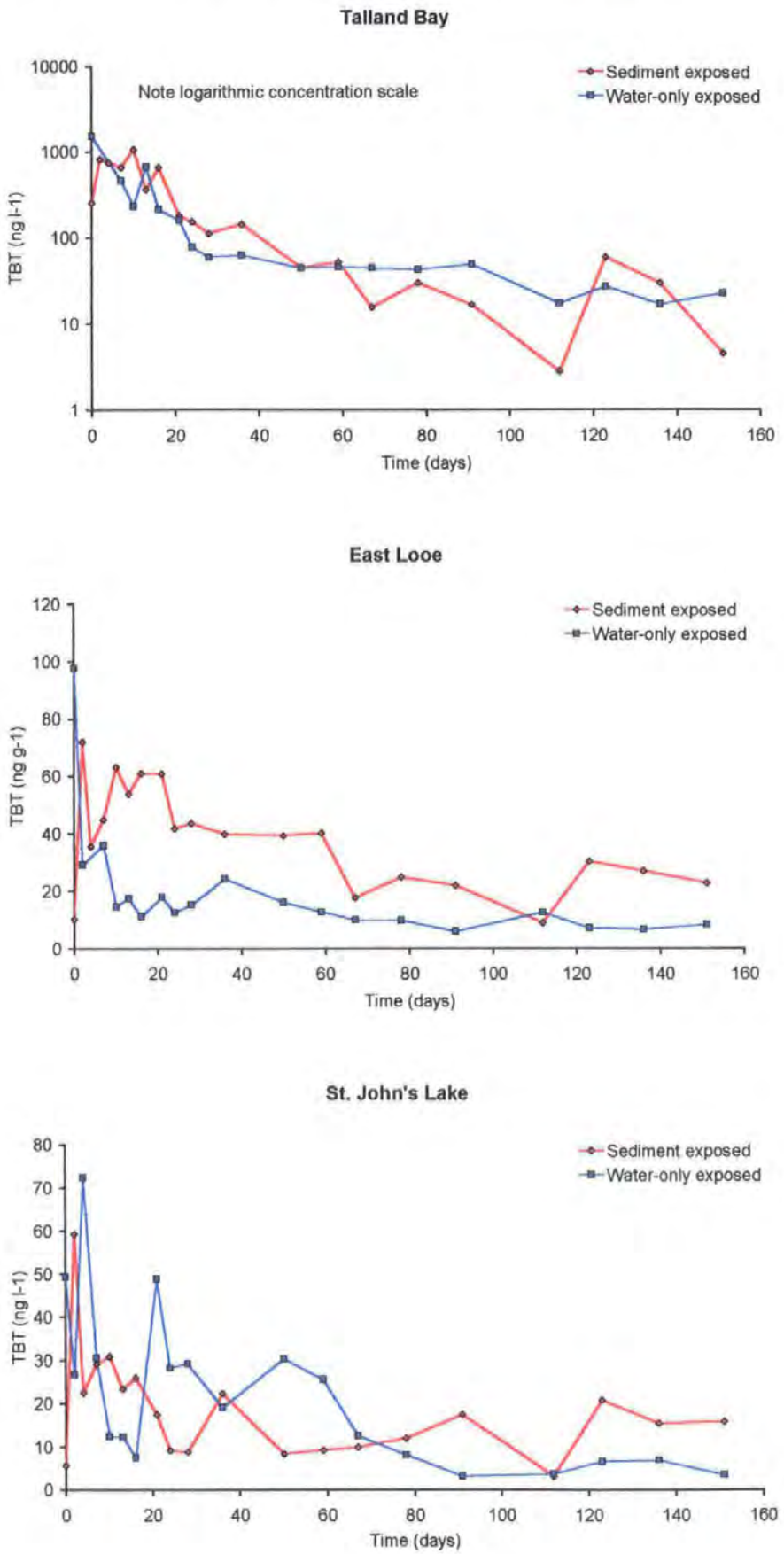
The results for TBT+DBT and TBT concentrations in sediments and water samples are presented in full in appendices 8 – 10. Average TBT concentrations in seawater to which *Hinia reticulata* were exposed prior to removal at each sampling interval are summarised in Table 6.1 and Figure 6.1. These results extend beyond the exposure period for *Hinia reticulata* described here since the systems were used as part of another experiment.

Table 6.1 Summary of dissolved TBT concentrations in sediment and water, and water-only exposures to different sediment types

(Values shown are means with standard deviations in parentheses)

Day	Exposure route	*n*	Talland Bay (ng l^{-1})	East Looe (ng l^{-1})	St. John's Lake (ng l^{-1})
10	Sediment	5	713.4 *(298.7)*	45.19 *(24.33)*	29.50 *(19.40)*
30		10	504.0 *(333.9)*	48.73 *(17.73)*	23.23 *(15.45)*
60		13	406.3 *(344.4)*	46.67 *(15.85)*	20.93 *(14.43)*
151		20	272.1 *(332.1)*	38.00 *(17.91)*	18.34 *(12.46)*
10	Water	5	738.0 *(685.6)*	44.31 *(36.72)*	38.26 *(23.13)*
30		10	423.9 *(487.1)*	27.94 *(27.40)*	31.74 *(20.09)*
60		13	322.2 *(443.2)*	25.37 *(23.96)*	30.17 *(17.80)*
151		20	209.2 *(370.0)*	19.18 *(20.54)*	21.83 *(18.43)*

Figure 6.1 Dissolved TBT concentrations in sediment and water, and water-only exposures to three different sediment types



Initial examination of these data for TBT concentrations in seawater showed that there were large differences in the TBT concentrations desorbed by the different sediment types, notably the Talland Bay sandy sediment, which initially exhibited very high TBT release rates. This however, was expected due to the low TBT K_d for this sediment, indicating a low adsorption capacity for TBT. As a result the variances for dissolved TBT concentrations were unequal and in order to compare the mean dissolved TBT concentrations to which *Hinia reticulata* had been exposed at each sampling interval, it was necessary to log transform the data prior to testing. Thus, ANOVA of log transformed data showed that for each sediment type, while there were no significant differences between the dissolved TBT concentrations in the sediment tank and in the water-only tank after 10, 30, 60 or 151 days ($P>0.05$, ANOVA), there were significant differences in the TBT concentrations desorbed by the different sediment types. The Talland Bay sediment desorbed significantly higher TBT concentrations than either East Looe or St. John's Lake sediments throughout the exposure period ($P<0.022$, ANOVA). Although mean TBT concentrations desorbed by the East Looe sediment were always slightly higher than the levels released by St John's Lake sediment (in the sediment exposure tanks, Table 6.1) these mean concentrations were not significantly different except when mean levels desorbed over 60 days were compared (46.67 ng l^{-1} for East Looe, and 20.93 ng l^{-1} for St John's Lake; $P=0.0492$, ANOVA). These TBT concentrations in the sediment tanks are compared in Figure 6.2 which emphasises the high levels desorbed by Talland Bay sediment in comparison with the other two sediment types.

Although each of the different sediments were initially spiked at approximately the same concentrations (1000ng g^{-1} TBT dry weight) TBT analyses before, during and after exposure to *Hinia reticulata* revealed some interesting trends. Data for these analyses are presented in Appendices 8 – 10. Initial TBT concentrations in the East Looe and St. John's Lake sediments were very similar at 1193 and 1172 ng g^{-1} dry weight respectively, while that for Talland Bay sediment was slightly lower at 882 ng g^{-1} dry weight. Most notably however, TBT concentrations in Talland Bay sediments decreased at a much faster rate than the levels in the other, fine grained sediments (Figure 6.3). East Looe and St. John's Lake sediment TBT concentrations decreased approximately linearly with time, and estimated half-times for loss were 313 and 383 days respectively, the slightly faster rate for East Looe sediments probably

Figure 6.2 TBT concentrations desorbed by East Looe, St. John's Lake and Talland Bay sediments

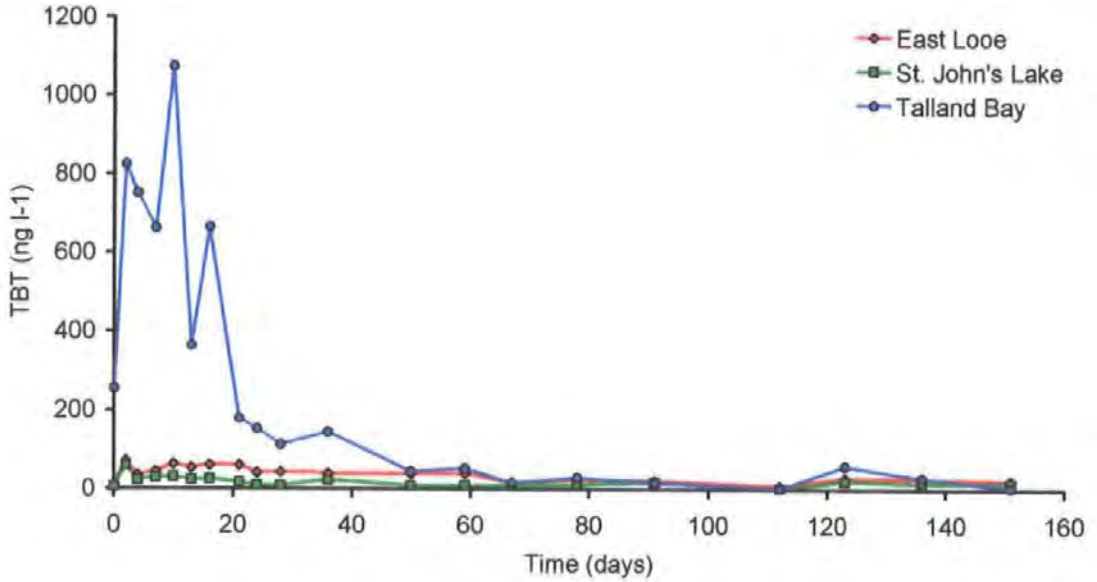
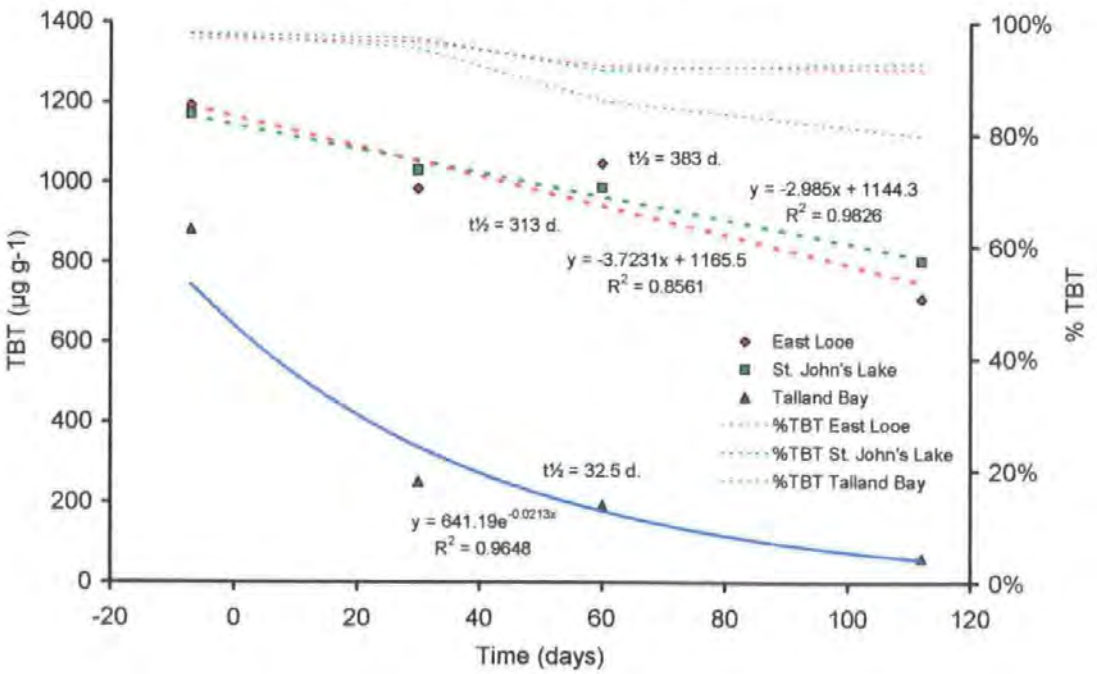


Figure 6.3 TBT concentrations in East Looe, St. John's Lake and Talland Bay sediments throughout exposure to *Hinia reticulata*



reflecting the slightly higher dissolved TBT concentrations noted earlier for this sediment. TBT concentrations in Talland Bay sediments however, decreased exponentially with time, with an estimated half-time of only 32.5 days (Figure 6.3) reflecting the lower K_d of this sediment, its reduced capacity for TBT sorption, and greater desorption of TBT. Thus while the two silty sediments exhibited only slight reductions in sediment TBT concentrations over the period during which *Hinia reticulata* were exposed (12.1 % reduction for East Looe and 15.7% for St. John's Lake sediments) Talland Bay sediments showed a 78.1% reduction in TBT burden over the same 60 day period.

6.3.2 Accumulation of TBT by *Hinia reticulata*

6.3.2.1 Whole animals

Data for accumulation of TBT by whole *Hinia reticulata* exposed to Talland Bay, East Looe, and St. John's Lake sediments and overlying waters are presented in Appendices 11 – 13 and summarised in Table 6.2.

Table 6.2 Summary data for accumulation of TBT by *Hinia reticulata* exposed to Talland Bay, East Looe and St. John's Lake sediments and overlying waters

(Data shown are means (n=10) with std. dev. in parentheses)

	Water-only exposed				Sediment and water exposed				% derived from sediment		
Day	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total	TBT+DBT	TBT
	Talland Bay										
10	3089.2 *(969.4)*	2283.8 *(597.2)*	1836.6 *(470.1)*	60.1%	12394.9 *(3123.9)*	9603.8 *(2428.6)*	7737.6 *(1924.3)*	62.7%	75.1%	76.2%	76.3%
30	3669.5 *(710.4)*	2857.9 *(668.8)*	2106.4 *(609.5)*	56.8%	11773.9 *(2455.9)*	9439.3 *(2390.6)*	6910.6 *(1842.0)*	58.3%	68.8%	69.7%	69.5%
60	2897.3 *(549.2)*	1820.0 *(312.5)*	1286.5 *(260.5)*	44.4%	No live animals remaining						
	East Looe										
10	236.4 *(47.4)*	166.7 *(36.9)*	135.1 *(33.2)*	56.9%	648.0 *(119.2)*	518.2 *(107.5)*	476.3 *(108.5)*	73.1%	63.5%	67.8%	71.6%
30	404.9 *(131.4)*	300.1 *(119.1)*	229.1 *(103.0)*	55.4%	1342.8 *(351.6)*	1112.5 *(357.9)*	989.8 *(323.9)*	73.4%	69.8%	73.0%	76.9%
60	459.1 *(71.6)*	308.4 *(56.0)*	221.6 *(43.9)*	48.2%	1752.5 *(369.7)*	1329.5 *(307.1)*	1143.3 *(250.8)*	65.2%	73.8%	76.8%	80.6%
	St. John's Lake										
10	222.0 *(54.0)*	167.6 *(45.1)*	145.7 *(43.2)*	65.1%	604.2 *(100.4)*	480.8 *(102.7)*	455.4 *(105.5)*	74.8%	63.3%	65.1%	68.0%
30	510.4 *(97.7)*	439.8 *(110.3)*	306.1 *(68.2)*	59.7%	1040.8 *(178.1)*	835.8 *(153.8)*	748.7 *(130.4)*	72.0%	51.0%	47.4%	59.1%
60	793.2 *(170.2)*	585.3 *(143.2)*	449.3 *(125.6)*	56.3%	1272.6 *(380.5)*	934.5 *(288.8)*	835.4 *(267.6)*	65.6%	37.7%	37.4%	46.2%

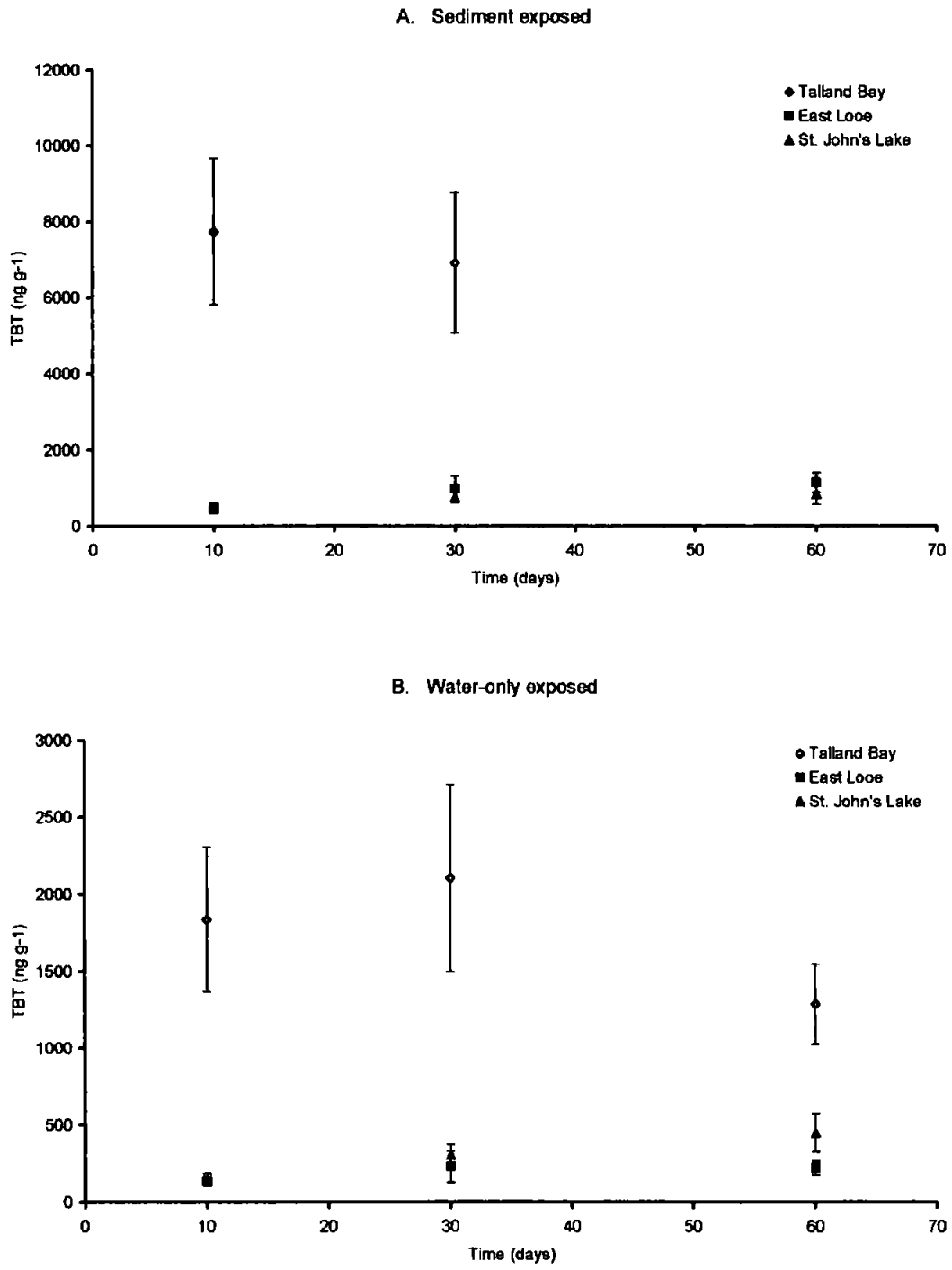
Unfortunately no *Hinia reticulata* survived after 60 days exposure to the Talland Bay sediment, although this itself may be significant and is discussed later.

Once again, initial data analysis showed significant heteroscedasticity between the results for the different sediment types and as a result all data were log transformed prior to analysis in order to equalise the variances in each case and satisfy the pre-requisite conditions for analysis of variance (Zar, 1984).

Comparison of the accumulated concentrations of 'total butyltins', 'TBT+DBT' and TBT in *Hinia reticulata* revealed almost identical patterns of accumulation, therefore only the results for TBT are presented here (Figure 6.4). The principal feature of these results was that *Hinia reticulata* exposed to TBT in the three sediment types accumulated significantly higher burdens ($P=0.00014$, ANOVA) from the sandy Talland Bay sediment than from the other, fine grained sediments at all sampling intervals (days 10 & 30, there being no survivors after 60 days exposure). An almost identical pattern was also seen in animals which were exposed only to the overlying waters. *Hinia reticulata* exposed to TBT that had leached from the Talland Bay sediment accumulated significantly higher concentrations of TBT in their whole body tissues, compared with the other two sediment types, after 10, 30 and 60 days exposure ($P=0.00013$, ANOVA). In contrast, comparison of TBT concentrations in *Hinia reticulata* exposed to TBT in East Looe or St. John's Lake sediments showed no significant differences at any time during exposure to sediments, while exposure to desorbed TBT in the overlying water resulted in a significant difference only after 60 days exposure (Figure 6.4B).

A further interesting feature of these results, shown in Figure 6.4 is that TBT concentrations in *Hinia reticulata* exposed to TBT in either sediments or water, continuously increased with time for East Looe or St. John's Lake sediments, but for Talland Bay sediments the very high concentrations accumulated after 10 days exposure to sediments, decreased with time, and was slightly (but not significantly) lower after 30 days exposure. Similarly, animals exposed only to TBT that had desorbed from this sediment, accumulated very high tissue concentrations after 10 days and increased slightly after 30 days, but had decreased after 60 days exposure. These reductions were almost certainly the result of the high initial release of TBT from Talland Bay sediments with resultant high initial TBT concentrations in the water and, subsequent

Figure 6.4 TBT concentrations accumulated by *Hinia reticulata* exposed to Talland Bay, East Looe and St. John's Lake sediments, and overlying waters



decline, together with concomitant decrease in the sediment TBT concentration shown in Figures 6.2 and 6.3.

Comparison of all (log transformed) butyltin concentrations accumulated by *Hinia reticulata* exposed to TBT in sediments and water, with those accumulated by animals exposed to the overlying water alone, showed significant differences at all sampling intervals for each sediment type ($P<0.0002$, ANOVA) as seen in earlier experiments. An exception was in the case of animals exposed to St. John's Lake sediments for 60 days, where no significant difference was apparent ($P>0.05$, ANOVA). These comparisons are summarised for each butyltin extract in Figures 6.5 –6.7. Accumulation of all butyltin species from East Looe and St. John's Lake sediments and overlying waters were logarithmic functions of exposure time as described earlier (Chapter 4) while accumulation by *Hinia reticulata* which had been exposed to Talland Bay sediments decreased with time, as already described.

Since there were no significant differences in TBT+DBT or TBT concentrations in the waters of sediment exposure tanks compared with water-only tanks, for each sediment type; nor any significant difference in the mean size or weight of animals, it was possible, once again, to estimate the proportion of the total body burden derived from the sediment, by subtraction (Table 6.2). This shows that for *Hinia reticulata* exposed to Talland Bay sediments, 75.1 – 76.3% of the accumulated tissue burdens were derived from the sediment after 10 days exposure, decreasing slightly to 68.8 – 69.7% after 30 days. East Looe sediments similarly, showed 63.5 – 71.6% of burdens to be sediment derived after 10 days exposure, increasing slightly to 73.8 – 80.6% after 60 days. St. John's Lake sediments however, showed 63.3 – 68% of accumulated levels to be sediment derived after 10 days but decreased gradually to 47.4 – 59.1% at 30 days, and only 37.4 – 46.2% after 60 days. A possible explanation for this decrease in relative importance of the sediment as a source of TBT with time may be revealed by considering the bioaccumulation factors for TBT in water-only exposed *Hinia reticulata* (Table 6.3).

Table 6.3 Mean bioconcentration factors for TBT in *Hinia reticulata* exposed to water overlying Talland Bay, East Looe and St. John's Lake sediments

Day	Talland Bay			East Looe			St. John's Lake		
	TBT in water (ng l^{-1})	TBT in *Hinia* (ng g^{-1})	Bioconc factor	TBT in water (ng l^{-1})	TBT in *Hinia* (ng g^{-1})	Bioconc factor	TBT in water (ng l^{-1})	TBT in *Hinia* (ng g^{-1})	Bioconc factor
10	738.0	1836.6	**2489**	44.3	135.1	**3049**	38.3	145.7	**3808**
30	423.9	2106.4	**3456**	27.9	229.1	**8200**	31.7	306.1	**9644**
60	322.2	1286.5	**3995**	25.4	221.6	**8735**	30.2	449.3	**14892**

Bioconc. factor = TBT conc. in *Hinia* (ng g^{-1}) divided by TBT conc. in water (ng l^{-1}+1000).

Figure 6.5 Total butyltin concentrations accumulated by *Hinia reticulata* from TBT in Talland Bay, East Looe and St. John's Lake sediments and overlying waters during 60 days exposure.

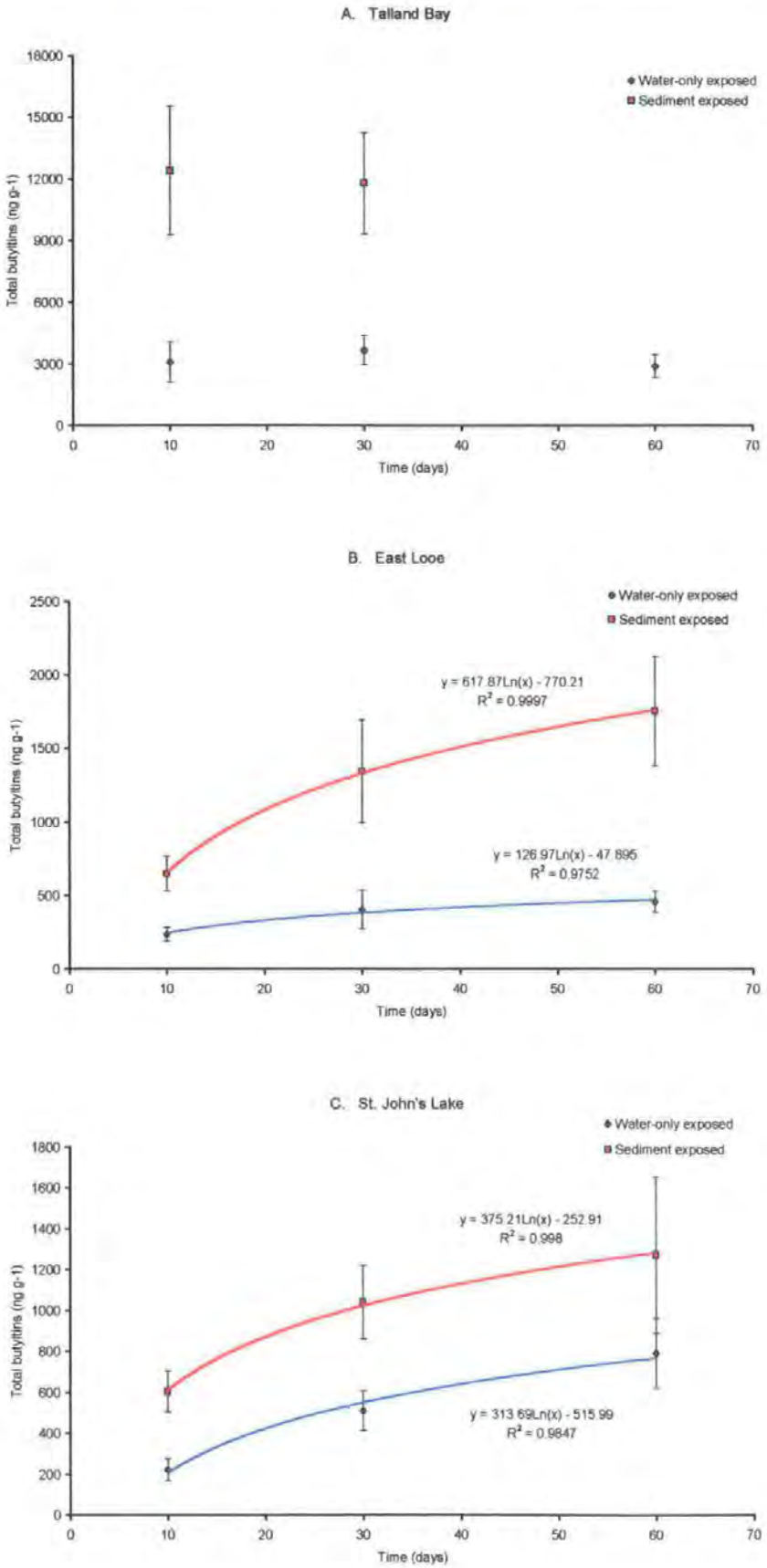


Figure 6.6 TBT+DBT concentrations accumulated by *Hinia reticulata* from TBT in Talland Bay, East Looe and St. John's Lake sediments and overlying waters during 60 days exposure.

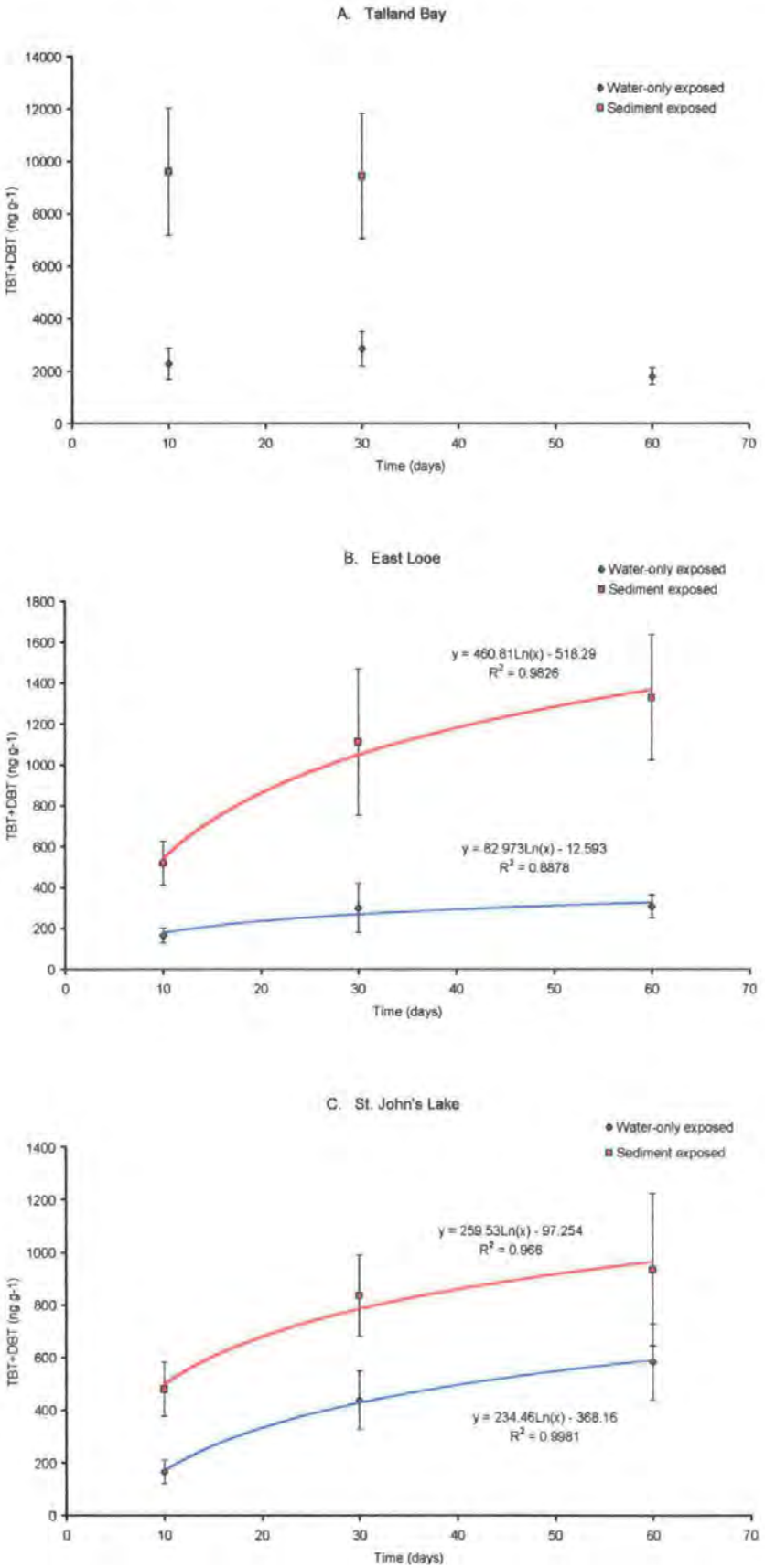
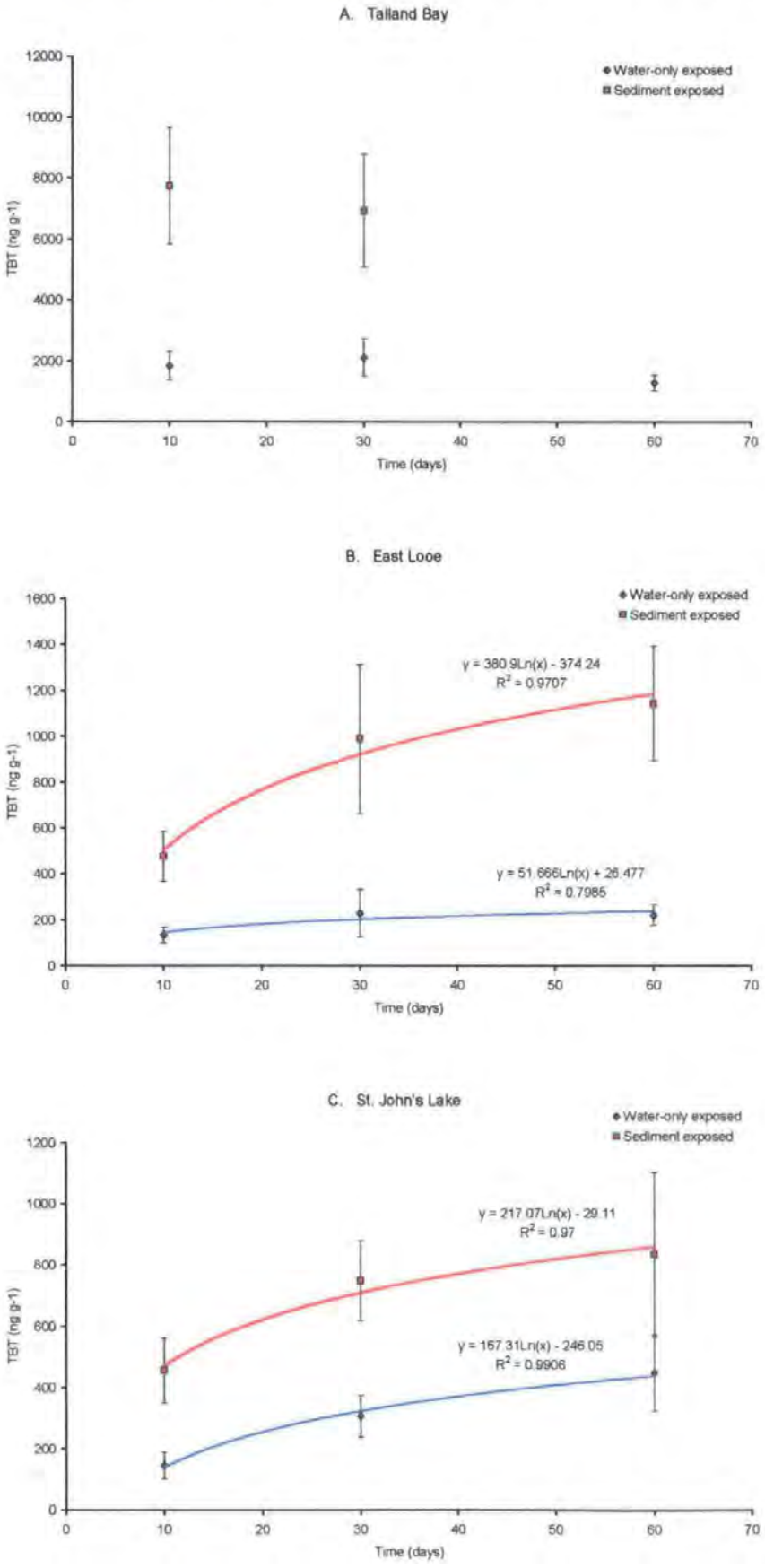


Figure 6.7 TBT concentrations accumulated by *Hinia reticulata* from TBT in Talland Bay, East Looe and St. John's Lake sediments and overlying waters during 60 days exposure.



This reveals that the TBT bioconcentration factors for *Hinia reticulata* exposed to seawater overlying St. John's Lake sediments were somewhat higher than for animals similarly exposed to the water overlying East Looe sediments, despite there being no significant difference in the dissolved TBT concentrations. This suggests that TBT bioaccumulation from the different sediments *and* the bioavailability of the TBT which has desorbed from them, may be modified by factors specific to those sediment types.

6.3.2.2 Tissue distribution of butyltins

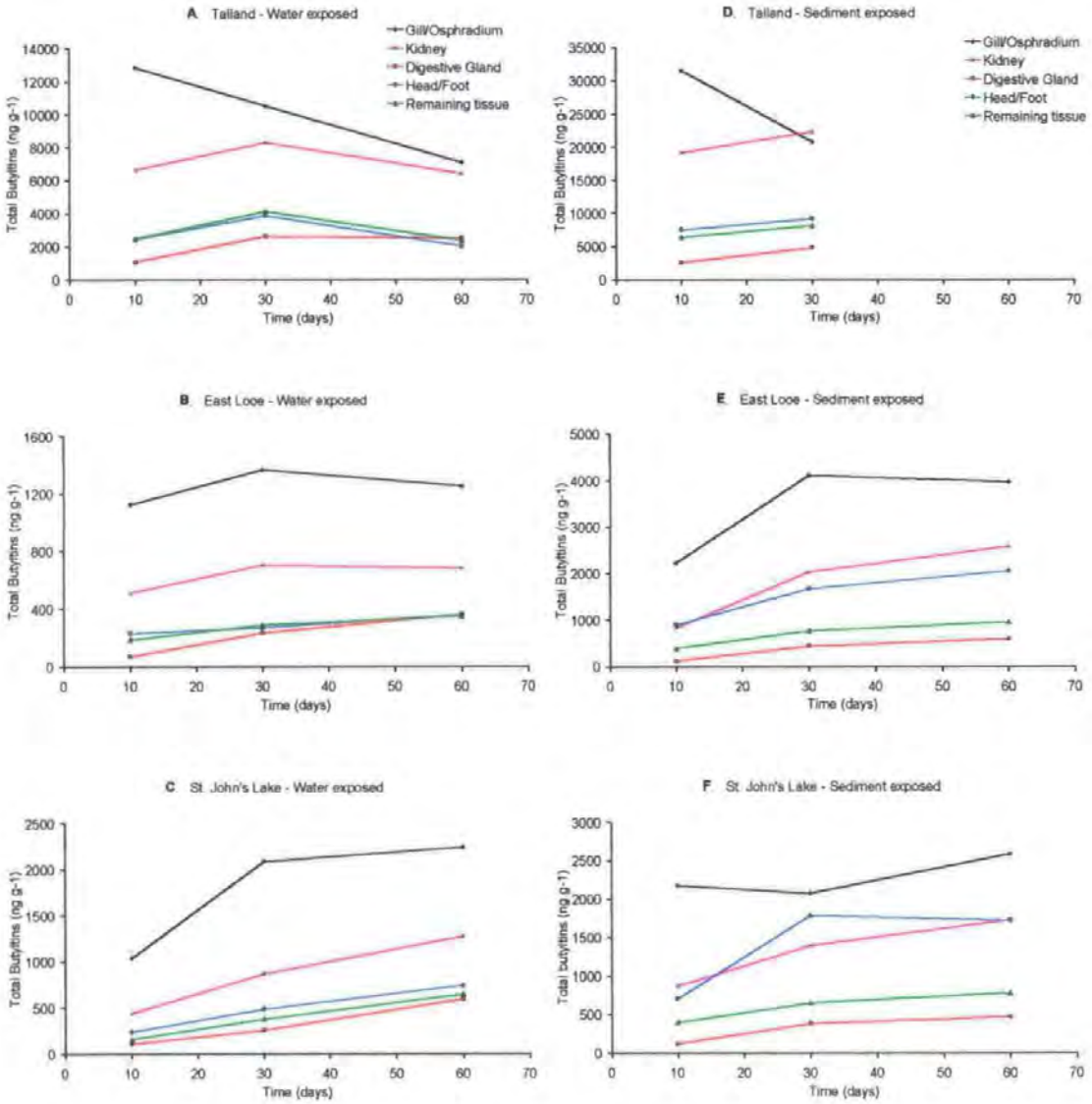
Data for mean concentrations of 'total', 'TBT+DBT' and TBT in pooled samples of gill/osphradium, kidney, digestive gland, head/foot and remaining tissues of *Hinia reticulata* are presented in Appendix 14. The changes in 'total butyltin' concentrations with time for sediment and water exposed and water-only exposed animals, for each sediment type are shown in Figure 6.8 and reveal some interesting differences.

Highest concentrations occurred in the gill/osphradium tissues across all exposure regimes and sediment types. Most notably, the total concentrations in the gill/osphradium (and all other tissues) of Talland Bay sediment, and water-only exposed animals were much higher than for the other sediment types, reflecting the significantly higher concentrations reported in the last section for whole body tissues. However, these concentrations decreased with time after 10 days exposure (Figure 6.8A and 6.8D), as a result of the decreasing TBT concentrations in the sediment and overlying water reported earlier. Most other exposures showed increases in total butyltin concentration in the gill/osphradium between the start of exposure and day 30, followed by a 'levelling off' in concentration by day 60.

The second highest total butyltin concentrations were seen in the kidney of all water-only exposed animals (Figure 6.8A-C) as previously reported in chapter 5, reflecting the importance of this organ in filtration of the blood, and excretion of metabolic products. All other tissues including the digestive gland, head/foot and the remaining tissues showed very similar total concentrations within water-only exposed animals for each sediment type.

Figure 6.8 Total butyltin concentrations in tissues of *Hinia reticulata* exposed to Talland Bay, East Looe and St. John's Lake sediments, and overlying waters.

(Values shown are from pooled samples *(n=10)* of each tissue type).



More noticeable differences in the pattern of accumulation in different tissues were seen for sediment exposed *Hinia reticulata*. For East Looe and St. John's Lake sediments, the concentration of total butyltins in the head/foot tissue of sediment exposed animals, was higher, compared with the digestive gland and remaining tissues than in water-only exposed animals, as reported in chapter 5. However, for Talland Bay sediment exposed animals, total butyltin concentrations in the head/foot remained similar to those measured in the digestive gland and the residual tissues, suggesting that for this sediment, TBT uptake across the exposed surface of the head/foot may not be as important a route compared with the East Looe or St. John's Lake sediments.

These data refer to the *concentrations* in the tissues, but, as highlighted in the preceding chapter, further insight into the importance of different uptake routes may be achieved by estimating the relative proportion of the total body burden contained in each tissue, and furthermore, by estimating the percentage of these tissue burdens that are attributable to uptake of TBT from the sediment and water phases. In order to complete these estimations it is necessary to make some simple assumptions:

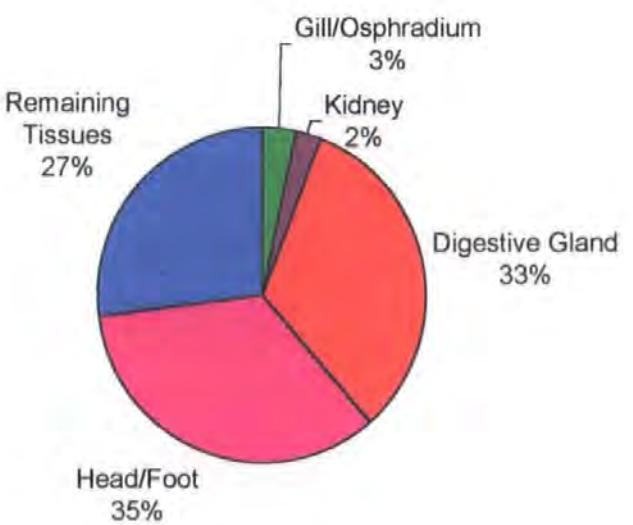
1. That for each sediment type, animals exposed to sediments and the overlying water obtain the same amount of TBT from the overlying water as those exposed to the water alone. Thus by subtraction, the difference is assumed to be due to uptake from the sediment.
2. To calculate the butyltin burdens in each tissue type, the dry weight of each tissue type was averaged from all *Hinia reticulata* used in the experiment. Thus, an 'average' *Hinia reticulata* has been estimated to conform to the composition shown in Table 6.4 and Figure 6.9.

Table 6.4 Dry weights of individual tissues of *Hinia reticulata* and their contribution to the total mass of the animal.

(Mean value based on 170 individuals, mean shell height 26.93mm ± 1.81mm)

Tissue	Dry weight (mg)	Proportion of total body mass (%)
Gill/Osphradium	6.1	3.46%
Kidney	4.2	2.39%
Digestive Gland	57.7	32.78%
Head/Foot	60.5	34.37%
Remaining tissue (predominantly hypobranchial gland)	47.5	26.99%
Total Body	176.0	100%

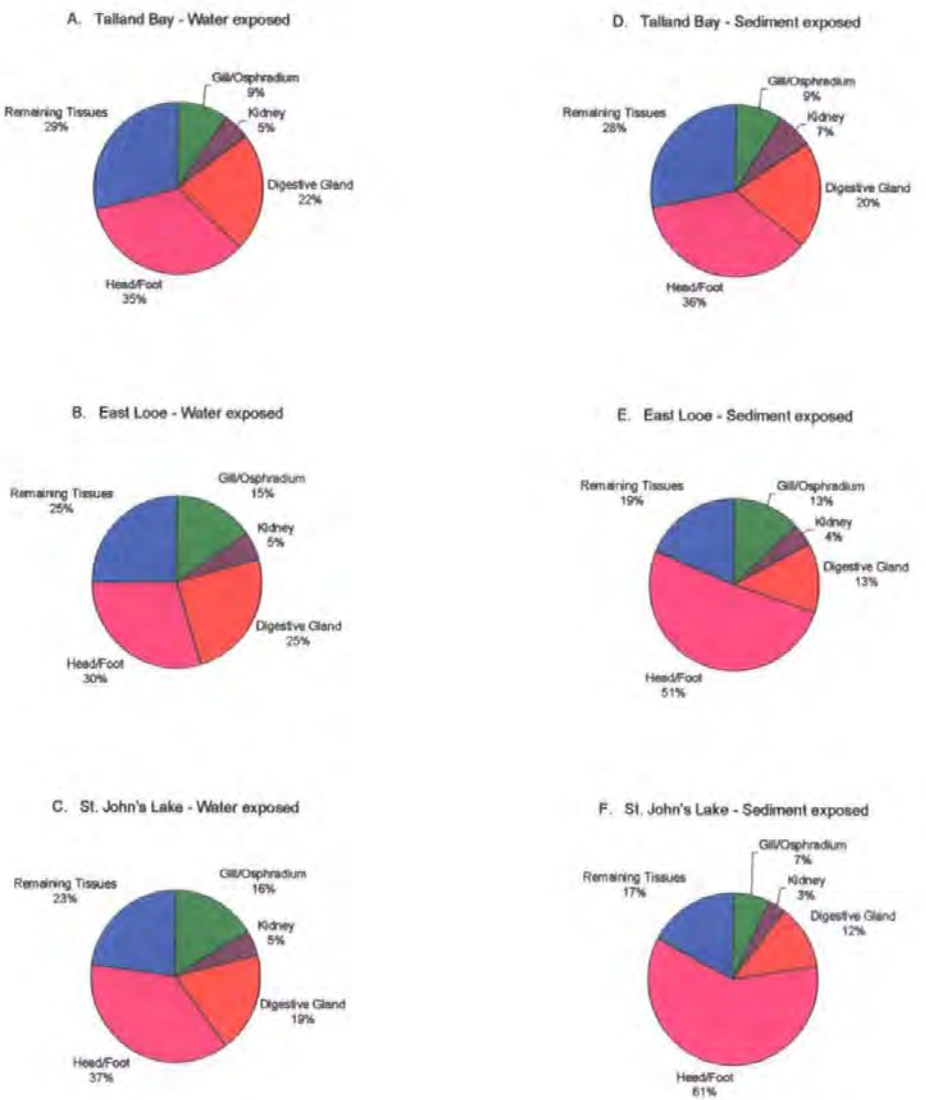
Figure 6.9 Tissues of *Hinia reticulata* as mean percentages of the whole animal on a dry weight basis.



Therefore, utilising these assumptions, butyltin burdens in each tissue have been estimated for *Hinia reticulata* exposed to each sediment type after 30 days exposure (the last time for which data were available for all sediments and exposure routes). In addition, the percentage of each tissue burden derived from the water has been estimated, together with the additional burden accumulated when *Hinia reticulata* was also exposed to TBT contaminated sediment. These data are presented in Appendix 15.

Comparison of 'total butyltin' burdens in the different tissues for each sediment and exposure (Figure 6.10) shows that for *Hinia reticulata* exposed to Talland Bay sediments, or the overlying water, there was very little difference in the proportional distribution of butyltins for each exposure route, although the actual burdens in those additionally exposed to sediments were higher, with the sediment estimated to contribute approximately 59 – 72% of the total burden (compared with 68.8% in whole animal tissues), depending on tissue type (Appendix 15). In contrast, East Looe and St. John's Lake sediments revealed very different distribution of total butyltins between water-only exposed animals and those which were also exposed to TBT-contaminated sediment. Principally, animals which were exposed to these sediments accumulated much higher proportions of their total body burden in the tissues of the head/foot compared to animals which had only been exposed to the overlying water (Figure 6.10). These increased burdens in the head/foot tissue of sediment exposed animals provide further evidence for uptake via this tissue when *Hinia reticulata* is in contact with TBT contaminated sediment. Conversely the absence of such a proportional increase in the head/foot tissues of *Hinia reticulata* exposed to Talland Bay sediments suggests that uptake across the head/foot may not be an important route for that type of sediment; similarity with water-only exposed animals, in that case suggesting that even in sediment-exposed animals uptake from the water (or pore water) may be the major route for TBT accumulation.

Figure 6.10 Distribution of 'total butyltins' between the tissues of *Hinia reticulata* exposed to Talland Bay, East Looe and St. John's Lake sediments, and the overlying water for 30 days.

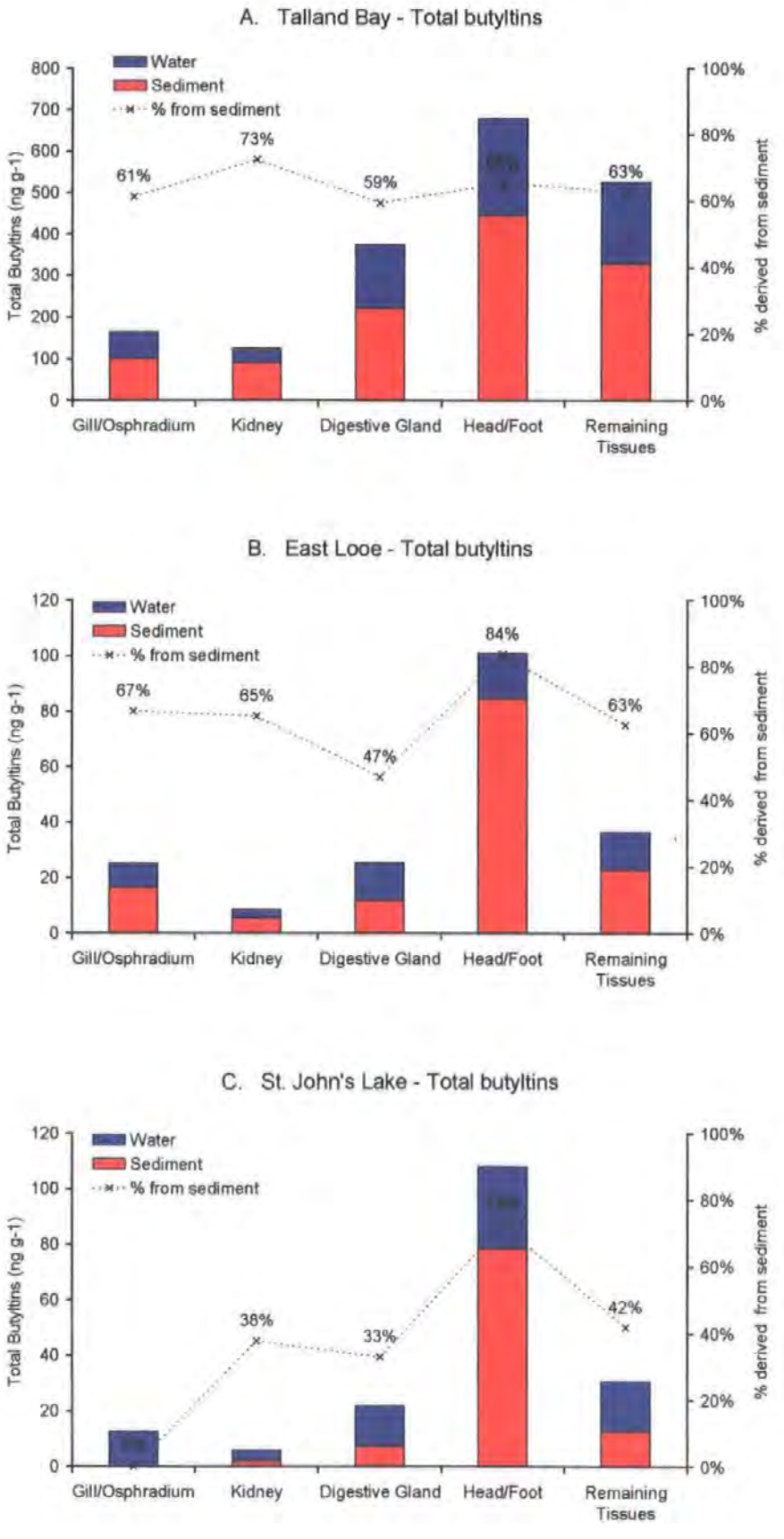


Considering some of the other tissues: the proportion of the body burden in the kidney showed little change across all sediments and exposures, although highest proportions were seen in Talland Bay sediment exposed animals (Figure 6.10D) which also had the highest accumulated whole body concentrations, and may indicate that in these animals the kidney was perhaps operating most strongly to remove TBT and its metabolites. The proportion of the total burden in the digestive gland showed little variation (19–25%) in water-only exposed animals, but in East Looe and St. John's Lake sediment exposed animals the proportion was lower, predominantly as a result of the much higher burdens in the head/foot tissue of these animals already discussed. However, data for 'total butyltins', 'TBT+DBT' and TBT in Appendix 15 shows that in every case the proportion of the body burden in the digestive gland decreases through the series 'total' > 'TBT+DBT > TBT (the only tissue for which such a decrease was apparent) which provides further evidence for metabolism of TBT in the digestive gland discussed in the previous chapter.

The relative proportions of the body burden present in the gill/osphradium shows some interesting differences between the different sediments and exposure routes. As already noted, Talland Bay sediment exposed *Hinia reticulata* show very little difference in tissue distributions compared with animals exposed only to TBT in the overlying water, the gill/osphradium containing 9% of the body burden in each case. *Hinia reticulata* which were exposed only to the water overlying the other two sediments however, showed 15-16% of the body burden to be in these tissues, although the actual amount was less than for Talland Bay exposed animals since the dissolved TBT concentrations were lower. Most notably though, the proportion in the gill/osphradium of St. John's Lake sediment exposed animals decreased to 7% compared with 16% in those exposed only to the overlying water. Possible explanations for these, and other differences may be revealed from the proportions in each tissue, which were estimated to be derived from the water, and, directly or indirectly, from the sediment (Appendix 15).

Figure 6.11 summarises the data in Appendix 15 for the estimated proportion of the 'total butyltin' burden in each tissue derived from the sediment or the overlying water after 30 days

Figure 6.11 Total butyltin burdens in tissues of *Hinia reticulata* exposed to Talland Bay, East Looe and St John's Lake sediments. Estimation of the proportions derived from sediments or the overlying water.



exposure. This shows that 59 – 73% of the accumulated 'total butyltins' in tissues of *Hinia reticulata* exposed to Talland Bay sediment were derived, directly or indirectly, from the sediment; the remainder being accumulated from the overlying water. This is in good agreement with the results for whole animals which showed 68.8 – 69.7% to be sediment derived after 30 days). However, as already shown there were no differences in the proportions of total butyltins in the different tissues under either exposure regime, suggesting no difference in uptake route, and that these 59 – 73% higher burdens in sediment exposed animals were still probably due to uptake from the water (including pore water?) in this sandy sediment, with a proven low capacity for adsorption of TBT (low K_d).

Hinia reticulata which had been exposed to East Looe sediments showed slightly greater variation in the proportions of tissue burdens estimated to have been derived from sediments and water. Most noticeable (Figure 6.11B) was the high proportion (84%) in the head foot attributable to accumulation from the sediment, suggesting that in this sediment type, uptake across the surface of the head/foot may be a more important uptake route than in the case of Talland Bay sediment.

St. John's Lake sediment exposed *Hinia reticulata* however, showed even greater variability, and after 30 days exposure total butyltin concentrations in the gill/osphradium of water-only exposed animals was slightly higher than in sediment exposed animals. Therefore estimates of the proportion of the burden in this tissue derived from the water were 100% and no excess could be attributed to additional accumulation from the sediment. In a similar fashion, the proportion of the tissue burdens in kidney, digestive gland and remaining tissues estimated to be sediment-derived, were all lower in this fine silty sediment with a high affinity for TBT (high K_d). The head/foot was the only tissue for St. John's Lake sediment exposed animals which showed a high proportion (73%) to have been accumulated from the sediment, once again suggesting that intimate contact of this structure with the sediment provides an additional uptake route for some sediment exposed animals.

6.4 Discussion

Results for the accumulation of TBT in whole body tissues of *Hinia reticulata* clearly demonstrate that the bioavailability of sediment-bound TBT varies between different types of natural sediments. Highest accumulations were consistently observed in animals exposed to the Talland Bay sediment, or its overlying water (which contained high concentrations of dissolved TBT that had desorbed from the sediment). Sediments such as that from Talland Bay, with a low TBT K_d (typically low organic content sediments with a high proportion of sand) have a low adsorption or 'binding capacity' for TBT, and would therefore be expected to desorb higher concentrations of TBT than sediments with higher partition coefficients such as the East Looe or St. John's Lake sediments. In addition, TBT which was adsorbed to the Talland Bay sediment would also have been expected to be less strongly bound to the sediment particles (and more readily removed) than in the other sediment types. Whether such a sandy, low organic content sediment would be expected to accumulate such high TBT concentrations (by adsorption from the water column) in the natural environment is uncertain but seems unlikely since low organic sands tend not to occur in the environments typical of high dissolved TBT concentrations such as poorly flushed marinas, docks and harbours. Nevertheless this sediment type perhaps serves as an extreme for the purpose of this experiment, and perhaps significantly, no animals survived exposure to this sediment for 60 days, indicating its acute toxic effect to *Hinia reticulata*.

The other natural sediment types employed were more typical of estuarine environments, although the TBT concentrations used were fairly high compared with reported concentrations in UK estuaries (Dowson et al., 1992; Langston and Burt, 1991; Langston et al., 1987) but were certainly not excessive. The mean TBT concentrations which desorbed from East Looe and St. John's Lake sediments during the course of this experiment were all <50 ng l^{-1} TBT, which although it may be considered high by present day environmental levels, is certainly a realistic concentration compared with levels reported in the UK a few years ago (Cleary, 1991; Cleary and Stebbing, 1987; Langston and Burt, 1991; Langston et al., 1987; Law et al., 1994; Waldock et al., 1987).

Accumulation of TBT by *Hinia reticulata* exposed to the East Looe or St. John's lake sediments (and their overlying waters) was considerably less than that accumulated from Talland Bay

sediment, and was expected, on the basis that these sediments with higher affinity for TBT (higher K_d) would retain sorbed TBT much more strongly than the low K_d sediment, making it less available for uptake. However, despite a two-fold difference in TBT equilibrium partition coefficient, East Looe (K_d = 8300 l kg^{-1}) and St. John's Lake (K_d = 16700 l kg^{-1}) sediments showed no significant difference in the TBT concentrations accumulated from them by *Hinia reticulata* throughout the exposure period (although trends in the data showed that slightly lower concentrations were present in St. John's Lake exposed sediments at each sampling occasion).

Comparison of TBT concentrations accumulated by sediment and water exposed *Hinia reticulata*, with concentrations accumulated by animals that had been exposed to the overlying water alone showed, for each sediment type, significantly higher concentrations in sediment-exposed animals. In general, it appeared that up to 80% of the TBT accumulated by sediment exposed animals could be attributed to additional uptake from the sediment phase, the remainder being derived from the overlying water, although there were some differences between the sediment types. *Hinia reticulata* which had been exposed to St. John's Lake sediment showed a gradual reduction with time, of the proportion of their burden estimated to be derived from the sediment and a gradually increasing proportion derived from the water. In fact the concentrations accumulated by water-only exposed animals were generally slightly higher than those in animals which had similarly been exposed to the water overlying East Looe sediment (although significant differences were only apparent after 60 days exposure). This appeared to be contrary to expectations, in that the sediment with the highest affinity for TBT appeared to have more available TBT in the overlying water despite there being no significant difference in the dissolved TBT concentrations between the sediment types. Evidently the effects of different sediment type may be more subtle than is explained simply by sorption criteria.

Analyses of TBT in the separate tissues of *Hinia reticulata*, which had been exposed to the different sediment types and their overlying waters, showed some interesting differences, which may indicate changing uptake routes for different sediment types. In the sandy, low K_d, Talland Bay sediment, although significantly higher body burdens were accumulated by animals exposed to the sediment in addition to the overlying water, there was no difference in the

distribution of TBT in the tissues in either exposure. This suggests that uptake in sediment exposed animals was essentially via the same route as in those exposed only to the water, there being no evidence of increased uptake through the head/foot tissue. I suggest that in this relatively coarse grained, low organic sediment, which had been demonstrated to have a low adsorptive capacity, the sorbed TBT was readily desorbed to the pore-water and, due to the greater porosity and permeability of such a sediment, higher concentrations of TBT were released to the overlying water. When *Hinia reticulata* were present in this sediment they caused considerable bioturbation and mixing of the sediment and pore water which released more of the weakly sorbed TBT to the water which was then available for uptake (from the water). Thus, each *Hinia reticulata* could effectively have been creating its own microcosm of TBT enriched water through its own burrowing activity. Therefore very high TBT concentrations in the gill/osphradium would result in transfer of TBT to the rest of the body via the blood, resulting in burdens in all tissues. These burdens approximated the distribution (as mass fractions) of these tissues as part of the whole animal (Compare figures 6.9 with 6.10A and 6.10D). Higher burdens in the gill and kidney perhaps indicating the high TBT levels in the blood from uptake of dissolved TBT and elimination through ultrafiltration respectively (though some excretion of more water-soluble metabolic products may also occur across the gill surface).

Hinia reticulata which had been exposed to the other sediments showed a markedly different distribution of TBT in their tissues, with much higher burdens in the head/foot tissue in comparison to animals in the overlying water. Unlike the Talland Bay sediment, these fine grained organic rich sediments had already been shown to exhibit a high affinity for TBT, further evidenced by their lower, and slower, release of TBT back to the overlying water by desorption than was apparent for the sandy sediment. I suggest that in these fine grained sediments the TBT concentrations in the pore water may be much higher than in the overlying water since low porosity and permeability of these sediments do not allow it to transfer so readily. Hence, the head/foot of burrowing *Hinia reticulata* would be in contact with TBT-rich pore water which could be taken up across its surface. In addition, the possibility exists that there could be some uptake through the direct contact of the tissue with the contaminated sediment particles, and if so, the contact area may be expected to increase as the sediment

particle size decreased. Notably, burdens in the head/foot of sediment exposed *Hinia reticulata* increased with decreasing grainsize for the sediments used here.

Measured concentrations of TBT in the overlying waters of East Looe and St. John's Lake sediments showed no significant differences during the exposure period, although the trend was for slightly higher levels in the East Looe system (this was expected on the basis of its lower K_d). Likewise the whole body concentrations of TBT in the water-only exposed animals showed no significant difference between the two sediment types, except after 60 days exposure, when, surprisingly, accumulated levels in St. John's Lake water-exposed animals were significantly higher than in East Looe exposed animals. Trends in the data had shown slightly higher (non-significant) TBT levels to occur in St John's Lake water exposed animals on most other sampling occasions. In contrast, comparison of sediment and water exposed *Hinia reticulata* for these two sediment types showed no significant difference at any time, although those exposed to East Looe sediments accumulated slightly higher whole body concentrations, possibly attributable to the slightly higher dissolved TBT concentrations in the overlying water. However, analysis of individual tissues of *Hinia reticulata* exposed to these two sediments and their overlying waters enabled the relative contributions of TBT from the water and the sediment to be estimated, and revealed differences for the East Looe and St. John's Lake sediments. Principally, *Hinia reticulata* which had been exposed to St. John's Lake sediments for 30 days were estimated to have received all of their accumulated butyltin burden in the gill/osphradium from the overlying water and none from the additional sediment exposure. Similar low sedimentary contributions were apparent in the kidney, digestive gland and remaining tissues for St John's Lake sediment exposed animal. Only the head/foot tissue showed a higher proportion of the total butyltin burden (73%) to be attributable to the sediment. In contrast, animals which had been similarly exposed to East Looe sediments were estimated to have received higher proportions of the total butyltin burden in the gill/osphradium (67%), kidney (65%), digestive gland (47%) and remaining tissues (63%) from the sediment, while 84% of the burden in the head/foot was estimated to be sediment-derived. Although there were no significant differences in total body burdens, this higher relative accumulation from sediment compared to water for the medium level K_d estuarine sediment suggests that perhaps as a result of the lower binding capacity of this sediment for TBT, its availability to *Hinia reticulata*

may be greater. There appears to be a slight conflict here however, in that *Hinia reticulata* exposed only to the water overlying St. John's Lake sediments accumulated more TBT, and exhibited higher bioconcentration factors than those exposed to the water overlying East Looe, although concentrations were significantly higher only after 60 days exposure and despite no difference in dissolved TBT concentrations. There could be several explanations for this, including the possibility that the gill irrigation rates for East Looe exposed animals could be reduced, possibly due to sediment exudates; or that the desorbed TBT could be associated with another dissolved component released by the sediment which reduced its availability for uptake. A further possibility is that the St. John's Lake sediment comprised a much higher proportion of very fine silt and clay sized particles than the slightly coarser East Looe sediment. As a result, it may have been that animals exposed within the St. John's Lake system took up some of this very fine particulate matter with the inhalant water so that contaminated sediment may have come into contact with the respiratory surfaces such that further desorption may have occurred prior to removal as pseudofaeces.

In summary, this investigation has demonstrated that differences in the bioaccumulation of TBT from different sediment types occurs, with greater accumulation by *Hinia reticulata* exposed to sediments with a low sorption capacity for TBT, which itself appears largely controlled through the sediment particle grainsize and the organic content. Similar results have been reported for other infaunal invertebrates and it was suggested that organic carbon association of TBT strongly influences the bioaccumulation and toxicity of TBT trough its regulation of interstitial water TBT concentrations (Meador et al., 1997). However, it appears that while sediment partition coefficients and sorption of TBT are important, models such as those for non-polar organic compounds, which relate bioaccumulation simply to sediment organic carbon and tissue lipids might not be straightforwardly applicable to organometallic compounds like TBT. Other factors including sediment porosity and permeability may also be important.

Accumulation of TBT by *Hinia reticulata* undoubtedly occurs across the respiratory surfaces and the compound is rapidly distributed to all other tissues. However, when additionally exposed to fine grained sediments additional uptake across the surface of the head/foot appears to be important, and may be increasingly important as the capacity of the sediment for

TBT (K_d) increases, although other sediment parameters may modify TBT availability or organism irrigation rates.

Further investigations with more varied sediment types would be needed resolve many of these uncertainties, but currently it may be most appropriate to conduct mesocosm studies similar to those described here if assessment of TBT availability from natural sediments is required.

7 General Discussion

The work described in this thesis has significantly extended the somewhat limited information available concerning the physicochemical behaviour and bioavailability of sediment-bound TBT. Consequently, this chapter considers two main aspects: Sediment-water partitioning of TBT and its environmental significance; and the bioaccumulation of TBT by *Hinia reticulata* with emphasis on the bioavailability of sediment-bound TBT. In addition, suggestions for future research are included which have emerged from the work presented in chapters 3 – 6.

7.1 Partitioning: Adsorptive and desorptive processes for TBT in sediments

Organisms that are exposed to a sediment-bound toxicant may be able to accumulate the compound by a variety of routes: by its release back into the overlying water; from the sediment interstitial water; and for deposit-feeding organisms, by ingestion of contaminated sediment (Anderson et al., 1987). Therefore the bioavailability of sediment-bound toxicants will be critically affected by the way that the compound partitions between sediments and water, and on the biology of the organism. A further complication for partitioning is that sediments differ greatly in their physicochemical parameters, and that sediment distribution in estuaries is variable and largely dependent on the hydrodynamic energy of the environment, together with the availability of component materials.

Previous studies on sediment-water partitioning of TBT (Harris and Cleary, 1987; Kram et al., 1989; Randall and Weber, 1986; Stang and Seligman, 1987; Unger et al., 1988; Valkirs et al., 1986a; Valkirs et al., 1987b) provided valuable information on TBT sorption, but may have limited environmental significance due to the procedures used in their determination, or the pre-treatment of the sediments employed (see chapter 3). The methods described in this thesis utilised robust and quantitative laboratory protocols with substrates in as near natural condition as practical, at TBT concentrations which were realistic for UK estuarine environments.

Results from this investigation showed that a high proportion of TBT in a sediment/water system is rapidly scavenged by the solid phase (60% after 10 minutes) in agreement with earlier reports (Harris and Cleary, 1987). Equilibrium partitioning however, appeared to take longer, probably as the result of TBT diffusion to internal surfaces of the sediment particles (Karickhoff, 1980). This rapid adsorption of TBT has a strong environmental significance in that

it demonstrates that dissolved TBT can be sequestered by particulates within a short time, such as that when bed sediments in estuaries are re-suspended at periods of maximum tidal flow.

Many of the published partition coefficients for TBT and sediments (see Table 3.1) were derived from experiments where the TBT concentrations used were considerably in excess of those encountered in the environment, even in the most severely contaminated situations. This investigation has revealed that the sorption isotherm for TBT on a natural sediment was non-linear and described a Freundlich-like dependence on its own concentration typical of ionizable organic solutes where solute/sediment interaction energies decrease as the higher energy adsorption sites gradually become filled when more solute is added (Podoll and Mabey, 1987). Thus, the value of the TBT partition coefficient (K_d)is not constant but decreases as the TBT concentration in seawater increases. As a result, K_d values which may have been calculated from experiments using high TBT doses, and which result in unrealistically high concentrations in sediments and seawater may not be reliably used as predictors of TBT behaviour in the environment. In contrast, the use of ^{14}C labelled TBT in this investigation, with its greater inherent sensitivity, has enabled values for the partition coefficient to be determined at environmentally realistic TBT concentrations.

Suspended sediment load was found to exert a limited influence on the partition coefficient across a range from 0.045 – 1.3 g l^{-1} suspended solids, with this 30-fold difference in suspended load resulting in only a 2-fold difference in K_d. However, there did appear to be a (non-significant) trend towards higher K_d values at very low levels of suspended solids. This may be the result of a transfer of solute binding material (dissolved, macromolecular or microparticulate) from the sediment to the water phase (Voice and Weber, 1985). The amount of this material would be proportional to the amount of sediment present so that the seawater phase could hold more TBT in solution as more sediment material becomes suspended.

If this solids effect occurs in the environment, there could be significant implications with respect to the distribution and fate of TBT in estuarine environments, based on the following argument. In most estuaries, TBT inputs over the past 10 - 15 years have resulted in its concentration in the bed sediments. These sediments effectively represent a high concentration of suspended solids. When they become re-suspended in the overlying water, by whatever

means (tidal currents, bioturbation, dredging) the effective concentration of these contaminated solids are reduced by several orders of magnitude. If there is indeed a resultant increase in the partition coefficient at very low suspended solid concentrations (<100mg l^{-1}), and, if TBT levels in the overlying water are higher than that determined by the new K_d (for this level of solids), more TBT may become sorbed to the solid phase, and ultimately be transported back to the bed sediment. Here the solids concentration is effectively increased once more, K_d decreases and TBT may desorb into the interstitial water, where its diffusive flow would be slower than in the overlying water, thus leading to greater retention time in the sediment/water system than if K_d was constant.

Clearly this hypothesis is simplistic and would of course be dependent upon the environmental concentrations of TBT in all phases and any kinetic limitations, together with the type and concentration of the sediment in consolidated and suspended form. Nevertheless, it perhaps illustrates the importance of partitioning criteria in understanding natural systems, and the effect on the bioavailability of contaminants to estuarine organisms.

Previously reported effects of salinity on TBT partitioning were limited, and conflicting (Harris and Cleary, 1987; Randall and Weber, 1986; Unger et al., 1987; Unger et al., 1988) but had often been determined under very artificial conditions. This investigation has determined the effect of salinity on adsorption and desorption of TBT onto natural sediments with natural water. All results showed that highest concentrations of TBT in the water column occurred at intermediate salinities between fresh water and pure seawater. This has considerable environmental significance and demonstrates that during partitioning highest dissolved TBT concentrations are likely to occur at salinities typical of estuarine environments. However, these differences in dissolved TBT concentrations were relatively small, and more than 94% of all TBT present remained particle associated at all salinities.

Although seawater is usually well buffered at around pH 8, greater pH ranges may be encountered in some estuaries as the result of acid or alkaline wastes such as mine effluents or decaying vegetation. This study has shown that the amount of TBT desorbed by sediments to the overlying water is highly pH dependent, with greatest release at high, or low pH levels.

Conversely, maximum sediment retention of TBT occurred at a neutral pH of 7.14, although once again most of the TBT present was particle associated at all pH levels tested.

In contrast to many of the previous studies of TBT partitioning, which have attempted to determine a value for the TBT sediment-water partition coefficient (K_d), this investigation hypothesised that the K_d value would be expected to vary with different sediment types, dependent upon a number of physicochemical parameters. Investigations with a range of natural sediments showed, as expected, the major determinants to be the grainsize distribution of the sediment, particularly the proportion of fine and very fine silts, together with the organic content; higher levels of these factors increasing the affinity of the sediment for TBT. However, these factors alone were not the only sediment parameters to exert an influence on TBT sorption, and it appears very likely that several other factors (possibly including the proportion and type of humic acids, or manganese content) may affect the partitioning of this ionizable organometallic solute.

Several of these investigations into short-term partitioning and the factors that affect it, demonstrated that TBT desorbs from sediment to the overlying water. Longer term monitoring of TBT-spiked sediment in a laboratory flow-through system showed that sediment-bound TBT is continuously released back into the overlying water at concentrations known to be sub-lethally toxic to several species, with a half-time for release in the order of 3-4 years. In addition, little degradation of TBT to lesser butylated compounds was apparent, suggesting that degradative processes in sediment may not be as important a route for 'loss' of TBT in the environment compared with its degradation in seawater, although this may well be different under natural environmental conditions where greater biotic and abiotic factors would be expected to influence sediment processes. Throughout the course of all experiments in this research programme, it has been repeatedly demonstrated that any processes which cause disturbance of TBT contaminated sediments (whether burrowing by *Hinia reticulata*, or collection of sediment samples for analysis) results in greater release of TBT to the overlying water. Translating this to the real world: dredging or other disturbance of sediments laden with TBT, such as those in harbours, marinas or shipyards could result in sudden release of TBT to the water column.

In summary, the work described in this thesis has shown TBT to be rapidly scavenged from the water column by suspended particulate materials, resulting in its incorporation into the sediment phase. This process cannot be described by a single value for a partition coefficient (K_d) and varies widely, depending on a range of factors. Among these, TBT concentration itself is important since it affects the degree of saturation of available sorption sites, while sediment type is arguably the major determinant governing the degree of sorption, which has been shown to be greatest in fine grained sediments with a high organic content. Salinity and pH affect the sorption of TBT to a lesser extent, probably through modulation of TBT speciation in water, although most TBT remains particle associated under most conditions of pH and salinity. Furthermore, the sorption process has been conclusively shown to be reversible, with TBT contaminated sediments releasing the toxicant back to the overlying water for long periods after inoculation.

In terms of natural estuarine sediments contaminated with TBT, this study has demonstrated that, in addition to acting as environmental sinks during periods when TBT-based antifouling paints were in widespread use, these sediments may also act as long-term reservoirs and secondary sources of TBT to the water column, and that changing environmental conditions (salinity, pH and degree of physical disturbance) will influence its release. However, although these influences are now better described, perhaps the safest approach to the assessment of a particular instance of environmental concern is to consider it on a site-specific basis using partitioning experiments similar to those described, since the effect of sediment types has only been investigated for relatively few sediments.

Thus, release of sediment-stored TBT back to the water has been assessed, but what is the significance of TBT that remains sediment-associated during these processes? Investigations using *Hinia reticulata* to assess the availability of sediment-bound and desorbed TBT to benthic fauna have revealed important facets of this subject.

7.2 Bioaccumulation of TBT by *Hinia reticulata*

Previous studies on *Hinia reticulata* have shown it to be a useful biological indicator of dissolved TBT in seawater using the RPS, or preferably VDS indices (Bryan et al., 1993b; Stroben et al., 1992a; Stroben et al., 1992b). Its occurrence on predominantly sedimentary

shorelines where *Nucella lapillus* is absent has proved complementary, and the fact that populations of *Hinia reticulata* do not appear to become sterilised confers an additional advantage in its use as a bioindicator of TBT. Analysis of TBT in *Hinia reticulata* collected from UK sites showed that tissue concentrations were significantly correlated to dissolved TBT concentrations, but that a significant TBT burden remained when dissolved TBT concentrations were extrapolated to zero, suggesting uptake from sediments or food (Bryan et al., 1993b). Uptake of TBT from the diet has already been shown to be an important accumulation route (Stroben et al., 1992a)

The work presented in chapters 4 – 6 of this thesis has described the accumulation, metabolism and loss of TBT by *Hinia reticulata*, and has undeniably demonstrated that sediment-bound TBT is available for uptake by some benthic organisms.

Initial experiments showed *Hinia reticulata* to be a quantitative indicator of dissolved (desorbed) and sediment-bound TBT, exhibiting bioconcentration factors for TBT in seawater of up to 30,000 in laboratory experiments, comparing well with previously reported values (Bryan et al., 1993b; Stroben et al., 1992a). When exposed to TBT in sediments *Hinia reticulata* accumulated significantly higher tissue burdens than when exposed only to the water, although the relative importance of sediment-bound TBT was shown to vary with its concentration. At low TBT concentrations in the sediment (68 ng TBT g^{-1} dry weight) only 31 – 50% of the accumulated body burden could be attributed to sediment-bound TBT, the rest being taken up from the water. At higher sediment TBT concentrations the relative contribution of sediment-bound TBT increased, with up to 87% being sediment derived, showing that the importance of sediment associated TBT increases with the degree of environmental contamination. This was demonstrated by the fact that enrichment factors for sediment exposed *Hinia reticulata* (TBT concentration in *Hinia* divided by TBT concentration in the sediment) increased from a factor of ~1.5 (at 68 ng TBT g^{-1}) to >3.0 (at 6000 ng TBT g^{-1}).

Experiments described in chapter 4 together with other observations showed that the direct ingestion of TBT contaminated sediment was unlikely to be an important route for accumulation in adult *Hinia reticulata* (although this has not been determined for juveniles). Instead, when exposed to TBT contaminated sediment, *Hinia reticulata* appears to accumulate TBT via

several routes. Dissolved TBT, released to the overlying water is taken up across the respiratory surfaces of the gill/osphradium (and probably also by exposed mantle tissue) and transported in the blood to all other tissues where it is accumulated (presumably in the lipid rich fraction of cells). However, TBT in the sediment and interstitial water appears to be accumulated directly via the surface of the head/foot; this large structure (for the size of the organism) presenting a considerable surface area in contact with the pore water and the sediment particles. Similar accumulation of metals across the head/foot of molluscs has previously been reported (Ireland, 1982; Ireland, 1983) and may be a topic for further investigation. In most cases *Hinia reticulata* exposed to TBT in sediments accumulated higher TBT burdens in the head/foot than comparable animals exposed only to desorbed TBT in the water, although it appears that in some sediments, in particular sandy sediments with a low K_d, *Hinia reticulata* may create its own microcosm of bioturbation, itself releasing more TBT to the water, which may be taken up from the inhalant water current. In this situation enhanced accumulation in the head/foot may not be apparent since uptake is predominantly from the water.

Accumulation of TBT occurs in all tissues of *Hinia reticulata* with highest burdens present in the head/foot, digestive gland and 'remaining tissues' (defined in this context as those left over when all other tissues including the gill/osphradium and kidney had been removed). Very little metabolism of TBT was apparent in the head/foot or 'remaining tissues', but consistent occurrence of 'non-TBT' compounds, including DBT was apparent in the digestive gland, and to a lesser extent in the kidney suggesting a role for these tissues in TBT metabolism by *Hinia reticulata*, most likely in a cytochrome P-450 MFO system which has been reported for several other mollusc species.

Depuration of TBT from *Hinia reticulata* was difficult to assess with confidence when the animals had been allowed to accumulate TBT by natural means, since variability in the initial burdens between individuals led to poor correlations during depuration. However, when *Hinia* were injected with a fixed dose of TBT, and depuration monitored for 3 months, the loss of TBT was found to follow an exponential trend with time, greatest loss occurring within the first few days. Further analysis showed that 'half-lives' for the loss were approximately 26 days for all butyltins present (determined from Optisolv extracts) but only 17.9 days for (hexane

extractable) TBT indicating additional 'loss' of TBT by debutylisation. These depuration rates for TBT are relatively fast compared to many marine gastropods, where previously reported values have ranged from 48 – 120 days (Bryan et al., 1987; Bryan et al., 1989) suggesting that body burdens in *Hinia reticulata* may be more responsive to changing environmental TBT levels than if uptake and depuration were slower. In practical terms this could enhance its value as a biomonitor of TBT in the environment compared with species that show reduced capacity for TBT metabolism and loss.

The bioavailability of TBT to *Hinia reticulata* has been shown to vary between different sediment types, both in terms of the quantity of TBT accumulated, and in its distribution between the body tissues.

Sediments with a low sorption capacity for TBT such as low organic content sandy sediments most readily release adsorbed TBT back to the interstitial and overlying water, so that animals exposed to this type of sediment can accumulate very high TBT burdens, principally from the water via the respiratory surfaces.

In contrast, sediments with a higher sorption capacity (K_d) bind TBT more strongly, and release less to the overlying water. In these types of sediment *Hinia reticulata* accumulated much higher TBT burdens in the head/foot; the proportion increasing as the K_d of the sediment increased. Therefore accumulation routes appear to differ with the sorption capacity of the sediment, as predicted (see Chapter 3).

The environmental consequences of this are that lower K_d sediments may present greater TBT bioavailability than those with a higher sorption capacity in the short-term. However, due to their low sorption capacity these coarser, low organic sediments would be unlikely to accumulate so much TBT in the first place. Fine grained, organic rich sediments however, with their much greater sorption capacity for TBT are capable of sequestering higher concentrations of TBT from the overlying water; releasing it more slowly after inputs cease, with a half-life in the order of years. These types of sediment represent a much longer-term reservoir of TBT as demonstrated in the desorption experiments (Chapter 3). In addition to this re-release of TBT by desorption (which is increased by any sediment/water mixing process) the TBT in the sediment can be accumulated via contact of organisms such as *Hinia reticulata* with the

sediment particles and pore water. It is very likely that other sediment-dwelling biota will be subject to this exposure route, while deposit-feeding organisms such as *Scrobicularia plana* will also be exposed via the ingestion of contaminated sediment particles (Langston and Burt, 1991).

However, sediments may also affect the bioavailability of TBT desorbed from them through the influence of other sediment components that may be released to the water. Sediment components such as humic and fulvic acid have been demonstrated to enhance the water solubility of some organic pollutants and pesticides (Chiou et al., 1987; Chiou et al., 1986) and may be capable of altering the availability of TBT. In addition, sediment components could also affect the uptake of dissolved TBT through reduction or increase of organism irrigation rates, thus altering the capacity for uptake across the respiratory surfaces.

7.3 Conclusions

The major conclusion from this investigation is that sediment-bound TBT is not locked up in a non-bioavailable form. Although TBT introduced into the water column prior (and subsequent to) the imposition of partial paint bans would have been rapidly sequestered by particulates, the sorption process has been shown to be reversible, with sub-lethally toxic concentrations being desorbed back to the overlying water when new inputs have ceased. Any processes which increase the mixing of sediment and water enhances this release of TBT.

Sorption is not a constant process, but varies with sediment type, and TBT concentration; and to a lesser extent with salinity, pH and possibly suspended particle concentration, although the majority of the TBT present in a sediment/water system remains sediment associated at all times. This study has shown little evidence for TBT degradation in sediments although this may be very different in the field, where biotic and abiotic processes could be different. Nevertheless, the half-life of sediment-bound TBT appears to be in the order of years, so that in many highly contaminated areas sediments should remain a cause for concern for many years to come.

The bioavailability of sediment-bound TBT may occur via exposure to desorbed TBT; by exposure to TBT in the sediment and pore water; and for deposit-feeding organisms, by ingestion of contaminated sediment. Accumulation of TBT by *Hinia reticulata* was found to be

greatest in sediments with a low sorption capacity for TBT, since TBT was most readily desorbed from this type of sediment. However, sediments of this type are unlikely to act as persistent sources of TBT in the environment for the very reason that they release TBT most readily. More muddy, organic rich sediments most typical of sheltered or enclosed estuarine environments have a higher capacity for TBT and represent longer-term TBT reservoirs. *Hinia reticulata* has been shown to accumulate high TBT burdens from this type of sediment, predominantly through contact with the sediment and its pore water, although accumulation via the overlying water is also important. Ingestion of sediment does not appear to be a major route for this species.

Rapid and efficient uptake of TBT by *Hinia reticulata* has been demonstrated, together with relatively rapid metabolism, largely within the digestive gland, such that TBT burdens in the tissues provide a responsive monitor of environmental TBT levels.

In combination these results suggest that *Hinia reticulata* could be a useful organism for the monitoring of both dissolved and sediment bound TBT in marine and estuarine environments, particularly if used as part of comprehensive studies employing other types of organism such as macro algae, deposit-feeding bivalves, filter-feeding bivalves, and sediment dwelling polychaetes.

7.4 Suggestions for further research

Several aspects of TBT sediment water partitioning require further study, including the effect of additional sediment types, and their sorption isotherms, this would be useful to extend the database for TBT partitioning, and lead to greater insight and understanding of what is clearly a complex physicochemical process.

The relevance of TBT in interstitial waters is in need of investigation, in terms of its significance as a medium for accumulation in biota, and how interstitial TBT concentrations compare with sediment burdens and the concentrations in overlying waters in different sediment types.

Further investigations of TBT partitioning at very low levels of suspended solids would be useful to determine whether the micro-scale cycling of TBT sorption/desorption (discussed in Chapter 3) is possible.

The use of *Hinia reticulata* as a bioindicator for sediment-bound contaminants such as heavy metals should be pursued further; particularly its accumulation via the head/foot appears worthy of greater investigation. There could be potential for the use of *Hinia reticulata* in mesocosm type studies of sediment-metal bioavailability due to its availability, size and robustness in experimental systems. It could also be employed (given CEFAS permission) in field transplant experiments to assess bioavailability of TBT, and possibly other contaminants, in estuarine sediments under natural conditions.

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Appendix 1 Data for accumulation of organotins by *Hinia reticulata* exposed to low, medium and high levels of TBT in estuarine sediment

Low-level Exposure									
Day		Water-exposed				Sediment and water exposed			
		Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT	Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
9	mean	25.16	16.82	18.56	74.4%	39.51	33.70	33.88	83.6%
	n	5	5	5	5	5	5	5	5
	std. dev.	4.05	3.36	2.57	9.1%	14.63	10.32	15.26	13.0%
	min.	21.77	12.93	14.92	63.1%	26.99	21.03	16.96	62.8%
	max.	31.09	21.43	21.47	85.5%	62.54	45.38	55.08	93.9%
16	mean	29.97	30.07	26.62	89.3%	62.33	58.95	55.42	86.4%
	n	5	5	5	5	5	5	5	5
	std. dev.	3.92	5.12	5.22	15.7%	14.66	12.63	23.93	14.0%
	min.	23.61	22.98	21.92	74.6%	52.37	50.81	43.72	78.6%
	max.	33.81	37.15	35.47	113.6%	88.26	81.38	98.19	111.2%
23	mean	39.80	25.82	23.28	58.4%	63.28	34.60	35.99	57.9%
	n	5	5	5	5	5	5	5	5
	std. dev.	7.28	7.22	6.35	10.5%	13.51	8.26	8.92	12.9%
	min.	31.46	16.56	18.36	48.4%	44.62	27.06	25.21	38.2%
	max.	47.87	34.99	33.45	75.8%	78.15	47.48	47.98	67.6%
30	mean	53.50	31.19	22.04	41.5%	80.97	63.86	55.82	68.1%
	n	5	5	5	5	5	5	5	5
	std. dev.	12.09	13.25	9.46	13.7%	26.05	27.46	24.43	16.4%
	min.	35.63	18.03	12.31	19.3%	57.48	35.45	28.74	41.6%
	max.	63.77	53.13	34.28	54.8%	123.69	109.46	91.34	82.0%
44	mean	57.37	47.83	29.76	52.2%	83.75	60.50	50.38	58.9%
	n	5	5	5	5	5	5	5	5
	std. dev.	13.09	14.74	9.31	12.4%	18.70	6.78	18.03	13.5%
	min.	39.42	28.06	18.30	34.1%	64.39	53.22	22.65	35.2%
	max.	70.88	69.28	41.89	66.2%	110.66	70.27	71.76	68.4%
74	mean	52.78	43.06	29.66	56.0%	106.01	81.14	64.68	61.0%
	n	5	5	5	5	5	5	5	5
	std. dev.	10.64	9.29	8.37	12.3%	23.20	16.16	13.87	4.1%
	min.	39.55	31.73	16.51	41.8%	76.54	62.75	43.08	56.3%
	max.	68.93	53.04	36.66	73.0%	139.12	93.40	79.74	66.2%

Continued

Appendix 1 (continued) Data for accumulation of organotins by *Hinia reticulata* exposed to low, medium and high levels of TBT in estuarine sediment

Medium-level Exposure									
Day		Water-exposed				Sediment and water exposed			
		Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
9	mean	89.2	73.8	59.3	66.5%	711.4	551.0	473.5	69.1%
	n	5	5	5	5	5	5	5	5
	std. dev.	15.6	12.2	11.4	5.1%	302.1	196.8	139.9	10.0%
	min.	75.6	59.5	49.2	61.6%	498.8	403.3	364.3	56.6%
	max.	115.0	92.5	78.7	74.4%	1222.0	865.2	691.4	82.9%
16	mean	137.2	122.0	95.0	69.8%	857.8	781.2	700.9	81.1%
	n	5	5	5	5	5	5	5	5
	std. dev.	40.5	40.0	28.8	8.0%	157.0	169.2	164.0	10.1%
	min.	92.7	76.1	70.8	60.3%	610.8	482.5	413.1	67.6%
	max.	198.8	186.4	144.9	77.2%	1027.1	899.1	806.2	95.8%
23	mean	213.3	152.6	109.0	51.8%	801.6	664.1	591.6	73.6%
	n	5	5	5	5	5	5	5	5
	std. dev.	63.9	35.8	25.6	4.8%	151.3	149.2	125.0	2.0%
	min.	170.2	124.4	88.3	46.4%	615.2	486.9	453.1	72.2%
	max.	326.6	215.2	153.5	56.9%	1013.2	890.6	780.4	77.0%
30	mean	231.5	187.1	137.1	59.6%	951.1	810.0	690.2	72.2%
	n	5	5	5	5	5	5	5	5
	std. dev.	36.5	30.0	21.3	7.7%	201.9	208.0	174.4	5.9%
	min.	185.7	142.4	112.3	49.2%	773.8	611.9	541.4	67.6%
	max.	285.0	220.0	159.0	68.1%	1250.4	1040.4	880.7	82.5%
44	mean	242.3	187.3	129.7	53.9%	1257.6	961.7	837.2	65.7%
	n	5	5	5	5	5	5	5	5
	std. dev.	19.6	14.5	19.4	9.5%	290.2	278.8	258.0	5.3%
	min.	218.3	167.9	100.8	37.3%	999.6	673.6	578.4	57.9%
	max.	270.0	205.9	148.3	61.3%	1713.9	1405.3	1238.8	72.3%
74	mean	330.7	237.9	175.8	53.2%	1580.7	1110.2	942.9	59.4%
	n	5	5	5	5	5	5	5	5
	std. dev.	48.4	43.9	28.6	4.7%	481.6	323.6	300.6	1.9%
	min.	278.7	186.4	144.2	49.7%	1132.7	823.5	655.6	57.9%
	max.	397.0	295.8	203.5	61.3%	2244.6	1536.8	1324.0	62.7%

Continued

Appendix 1 (continued) Data for accumulation of organotins by *Hinia reticulata* exposed to low, medium and high levels of TBT in estuarine sediment

High-level Exposure									
Day		Water-exposed				Sediment and water exposed			
		Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
9	mean	1381	1137	907	65.4%	8856	7517	6602	75.1%
	n	5	5	5	5	5	5	5	5
	std. dev.	310	298	251	8.6%	3056	2302	2026	6.9%
	min.	1074	804	619	56.0%	5674	4692	4060	67.9%
	max.	1825	1500	1242	76.9%	13887	10765	9431	86.2%
16	mean	2599	2117	1635	63.8%	12770	11744	9750	77.1%
	n	5	5	5	5	5	5	5	5
	std. dev.	878	521	453	3.5%	2379	1629	1397	6.5%
	min.	2004	1754	1348	59.0%	9537	9917	8149	67.6%
	max.	4097	3006	2416	68.3%	15788	14214	11916	85.5%
23	mean	3642	2907	2265	61.9%	15069	12249	10137	67.0%
	n	5	5	5	5	5	5	5	5
	std. dev.	584	505	479	5.3%	5262	4143	3717	7.3%
	min.	3166	2366	1716	54.2%	9672	7497	5921	59.7%
	max.	4545	3627	2897	67.3%	22040	17440	14552	76.9%
30	mean	4480	3760	2871	64.4%	17972	14669	12384	68.8%
	n	5	5	5	5	5	5	5	5
	std. dev.	270	174	147	7.0%	3114	2538	2381	4.8%
	min.	4090	3545	2675	58.2%	14105	10748	8807	62.4%
	max.	4705	4016	3056	74.7%	20952	17232	14926	74.2%
44	mean	7394	6583	5338	71.4%	15477	12794	10305	66.7%
	n	5	5	5	5	5	5	5	5
	std. dev.	1886	2390	2136	20.4%	3157	2740	2042	3.9%
	min.	5830	4769	3598	54.8%	11812	9823	7863	61.2%
	max.	10599	9561	7982	106.6%	20464	17205	13437	71.9%
74	mean	8948	6847	5283	58.6%	18259	13487	9689	52.7%
	n	5	5	5	5	5	5	5	5
	std. dev.	1628	1538	1266	6.1%	4900	4514	3501	10.8%
	min.	6415	4628	3462	54.0%	12741	9011	5963	36.9%
	max.	10730	8156	6729	68.9%	24770	19751	14524	65.8%

Appendix 2 Summary of 2-way ANOVA and Tukey multiple comparisons for effects of exposure and time on 'total butyltin' concentrations accumulated by *Hinia reticulata*.

(All data were log transformed)

Summary of all Effects

	df Effec	MS Effect	df Error	MS Error	F	p-level
Exposure	5	30.492	144	0.0101	3022.402	0
Time	5	0.869	144	0.0101	86.106	0
Exposure &	25	0.035	144	0.0101	3.421	1.8E-06

Tukey HSD test
Probabilities for Post Hoc Tests
INTERACTION: Exposure x Time

Exposure		L. Sed.	L. Sed.	L. Sed.	L. Sed.	L. Sed.	L. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.
	Day	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74
L. Sed.	9																																				
L. Sed.	16	n.s.																																			
L. Sed.	23	n.s.	n.s.																																		
L. Sed.	30	0.00035	n.s.	n.s.																																	
L. Sed.	44	0.00008	n.s.	n.s.	n.s.																																
L. Sed.	74	0.00003	n.s.	n.s.	n.s.	n.s.																															
M. Sed.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																														
M. Sed.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																													
M. Sed.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																												
M. Sed.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.																											
M. Sed.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.01423	n.s.	n.s.	n.s.																										
M. Sed.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.02438	0.00371	n.s.	n.s.																									
H. Sed.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																								
H. Sed.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																							
H. Sed.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																						
H. Sed.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00028	n.s	n.s.																					
H. Sed.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.02727	n.s.	n.s.	n.s.																				
H. Sed.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00026	n.s.	n.s.	n.s.	n.s.																			
L. Wat.	9	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																		
L. Wat.	16	n.s.	0.00050	0.00031	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s															
L. Wat.	23	n.s.	n.s.	n.s.	0.00147	0.00029	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s														
L. Wat.	30	n.s.	n.s.	n.s.	n.s.	n.s.	0.00144	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00028	0.04709	n.s.													
L. Wat.	44	n.s.	n.s.	n.s.	n.s.	n.s.	0.01196	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00005	0.00726	n.s.	n.s.												
L. Wat.	74	n.s.	n.s.	n.s.	n.s.	n.s.	0.00117	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00032	n.s.	n.s.	n.s.	n.s.											
M. Wat.	9	0.00004	n.s.	n.s.	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00005	n.s.	n.s.	n.s.											
M. Wat.	16	0.00003	0.00010	0.00015	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00003	n.s.										
M. Wat.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00140	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	n.s.								
M. Wat.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00007	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.						
M. Wat.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.01909	n.s.	n.s.			
M. Wat.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00062	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.		
H. Wat.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00085	n.s.	n.s.	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	
H. Wat.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00077	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.01312
H. Wat.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00004	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	
H. Wat.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.03040
H. Wat.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	0.00151	0.00003	0.00022	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
H. Wat.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	0.00099	n.s.	0.00100	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003

Appendix 2 (continued) Summary of 2-way ANOVA and Tukey multiple comparisons for effects of exposure and time on 'TBT+DBT' concentrations accumulated by *Hinia reticulata*.

(All data were log transformed)

Summary of all Effects

	df Effect	MS Effect	df Error	MS Error	F	p-level
Exposure	5	31.61	144	0.01221	2588.41	0
Time	5	0.75766	144	0.01221	62.0414	0
Exposure &	25	0.04319	144	0.01221	3.53636	9.2E-07

Tukey HSD test
Probabilities for Post Hoc Tests
INTERACTION: Exposure x Time

Exposure		L. Sed.	L. Sed.	L. Sed.	L. Sed.	L. Sed.	L. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.
	Day	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74
L. Sed.	9																																				
L. Sed.	16	n.s.																																			
L. Sed.	23	n.s.	n.s.																																		
L. Sed.	30	n.s.	n.s.	n.s.																																	
L. Sed.	44	0.04634	n.s.	n.s.	n.s.																																
L. Sed.	74	0.00004	n.s.	0.00009	n.s.	n.s.																															
M. Sed.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																														
M. Sed.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																													
M. Sed.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																												
M. Sed.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.																											
M. Sed.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.																										
M. Sed.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00488	n.s.	n.s.	n.s.	n.s.																									
H. Sed.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																								
H. Sed.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																							
H. Sed.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																						
H. Sed.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00812	n.s.	n.s.																					
H. Sed.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.																				
H. Sed.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.																			
L. Wat.	9	0.01482	0.00003	0.00454	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																		
L. Wat.	16	n.s.	0.01518	n.s.	0.00735	0.00590	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																	
L. Wat.	23	n.s.	0.00012	n.s.	0.00007	0.00006	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																
L. Wat.	30	n.s.	0.00995	n.s.	0.00471	0.00376	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.															
L. Wat.	44	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.														
L. Wat.	74	n.s.	n.s.	n.s.	n.s.	n.s.	0.03318	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	n.s.	n.s.	n.s.	n.s.												
M. Wat.	9	0.00028	n.s.	0.00101	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00003	0.00004	n.s.	n.s.											
M. Wat.	16	0.00003	0.00659	0.00003	0.01370	0.01660	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00003	n.s.											
M. Wat.	23	0.00003	0.00004	0.00003	0.00004	0.00004	0.03821	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00431	n.s.								
M. Wat.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00013	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	n.s.	n.s.						
M. Wat.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00010	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	n.s.	n.s.	n.s.				
M. Wat.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00032	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00778	n.s.	n.s.	n.s.			
H. Wat.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00227	n.s.	n.s.	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003			
H. Wat.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00041	0.02013	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.03849	
H. Wat.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.
H. Wat.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.02124	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.
H. Wat.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	0.04536	0.04399	0.00014	0.00730	0.00377	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
H. Wat.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	0.00094	0.03764	0.02125	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003

Appendix 2 (continued) Summary of 2-way ANOVA and Tukey multiple comparisons for effects of exposure and time on TBT concentrations accumulated by *Hinia reticulata*.

(All data were log transformed)

Summary of all Effects

	df Effect	MS Effect	df Error	MS Error	F	p-level
Exposure	5	31.4330	144	0.01541	2039.52	0
Time	5	0.50218	144	0.01541	32.5835	4E-23
Exposure &	25	0.05375	144	0.01541	3.4877	1.2E-06

Tukey HSD test
Probabilities for Post Hoc Tests
INTERACTION: Exposure x Time

Exposure		L. Sed.	L. Sed.	L. Sed.	L. Sed.	L. Sed.	L. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	M. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	H. Sed.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	L. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	M. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.	H. Wat.
	Day	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74	9	16	23	30	44	74
L. Sed.	9																																				
L. Sed.	16	n.s.																																			
L. Sed.	23	n.s.	n.s.																																		
L. Sed.	30	n.s.	n.s.	n.s.																																	
L. Sed.	44	n.s.	n.s.	n.s.	n.s.																																
L. Sed.	74	0.03446	n.s.	n.s.	n.s.	n.s.																															
M. Sed.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																														
M. Sed.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																													
M. Sed.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																													
M. Sed.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.																											
M. Sed.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.																										
M. Sed.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.																									
H. Sed.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																								
H. Sed.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																							
H. Sed.	23	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																						
H. Sed.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.																					
H. Sed.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.																				
H. Sed.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.																			
L. Wat.	9	n.s.	0.00004	n.s.	0.00004	0.00014	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003																	
L. Wat.	16	n.s.	n.s.	n.s.	n.s.	n.s.	0.00065	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.																	
L. Wat.	23	n.s.	0.00202	n.s.	0.00281	0.02304	0.00004	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.																
L. Wat.	30	n.s.	0.00015	n.s.	0.00020	0.00215	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.															
L. Wat.	44	n.s.	n.s.	n.s.	n.s.	n.s.	0.00041	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.														
L. Wat.	74	n.s.	n.s.	n.s.	n.s.	n.s.	0.00527	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.													
M. Wat.	9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00476	0.00012	0.00004	0.03086	0.03018												
M. Wat.	16	0.00003	n.s.	0.00006	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00002	n.s.										
M. Wat.	23	0.00003	0.03354	0.00003	0.02558	0.00317	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.							
M. Wat.	30	0.00003	0.00011	0.00003	0.00008	0.00004	0.01202	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00189	n.s.	n.s.			
M. Wat.	44	0.00003	0.00041	0.00003	0.00029	0.00005	0.03850	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00701	n.s.	n.s.	n.s.		
M. Wat.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00006	0.00008	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.	
H. Wat.	9	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	
H. Wat.	16	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00147	0.00005	0.00108	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
H. Wat.	23	0.00009	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00004	0.00041	0.00004	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
H. Wat.	30	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00571	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
H. Wat.	44	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	0.00055	0.04075	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00363	n.s.	
H. Wat.	74	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	n.s.	n.s.	n.s.	0.00108	n.s.	n.s.	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00192	n.s.	n.s.

Appendix 3 Butyltin concentrations in whole tissues of *Hinia reticulata* during depuration after uptake from sediments and water.

Exposure regime	Day	Size (mm)	'Total' butyltins (ng g^{-1})	'TBT + DBT' (ng g^{-1})	'TBT' (ng g^{-1})	%TBT
Low water	0	27.8	27.75	6.06	5.30	19.11%
		27.3	35.05	45.99	25.40	72.47%
		25.9	28.03	5.81	3.48	12.40%
		27.8	49.46	15.94	13.48	27.26%
		27.5	23.77	7.06	2.21	9.28%
	7	26.6	35.89	7.22	1.32	3.68%
		26.1	32.17	8.52	6.49	20.19%
		24.7	29.84	n.d.	n.d.	
		25.7	28.14	19.33	9.44	33.55%
		26.8	27.45	10.09	2.50	9.09%
	14	28.2	24.33	15.11	4.82	19.83%
		26.7	27.12	4.91	3.05	11.24%
		24.7	27.50	n.d.	n.d.	
		29.0	22.41	9.11	6.13	27.34%
		25.2	29.54	n.d.	10.55	35.70%
	28	26.4	22.14	6.58	0.60	2.69%
		23.9	28.05	8.37	16.93	60.34%
		25.4	31.58	n.d.	n.d.	
		23.5	36.54	n.d.	7.24	19.82%
		24.7	22.97	1.66	n.d.	
	35	26.6	28.06	9.78	8.46	30.16%
		25.7	23.87	4.68	n.d.	
		26.8	27.46	12.22	n.d.	
		24.7	40.57	3.90	9.21	22.70%
		24.0	28.98	9.66	7.17	24.74%
		25.1	20.11	12.56	n.d.	
Low sediment	0	27.3	100.17	86.24	71.42	71.30%
		27.2	97.95	75.09	62.46	63.77%
		28.5	91.72	63.06	39.38	42.93%
		25.2	82.82	56.71	40.89	49.37%
		24.6	147.91	100.55	63.21	42.74%
	7	28.6	91.66	67.72	56.92	62.10%
		29.9	71.79	39.45	35.76	49.81%
		25.2	133.19	94.04	68.14	51.16%
		23.1	91.71	73.02	64.26	70.06%
		29.9	99.46	69.43	51.79	52.07%
	14	26.1	122.41	81.49	52.53	42.91%
		28.5	66.78	52.15	34.69	51.94%
		23.7	114.80	53.77	76.35	66.51%
		30.3	60.45	29.79	29.26	48.40%
		25.1	83.12	56.10	61.17	73.59%
	28	26.1	60.85	24.69	19.62	32.24%
		24.9	82.07	43.71	17.93	21.85%
		27.9	106.26	77.63	53.88	50.70%
		28.2	70.13	37.13	30.56	43.58%
		24.0	99.62	61.57	25.81	25.90%
	35	25.3	82.07	39.87	41.41	50.46%
		28.6	55.60	28.95	18.07	32.50%
		26.5	89.44	42.86	32.64	36.50%
		28.4	60.36	34.15	27.46	45.50%
		27.2	83.84	57.07	20.34	24.26%
		28.0	78.35	40.38	15.82	20.20%

Continued

Appendix 3 (continued) Butyltin concentrations in whole tissues of *Hinia reticulata* during depuration after uptake from sediments and water.

Exposure regime	Day	Size (mm)	'Total' butyltins (ng g^{-1})	'TBT + DBT' (ng g^{-1})	'TBT' (ng g^{-1})	%TBT
Medium water	0	29.5	292.48	200.28	140.02	47.87%
		29.1	279.48	174.69	98.41	35.21%
		27.4	308.44	198.47	136.66	44.31%
		26.3	230.63	147.80	106.70	46.27%
		28.2	310.30	215.30	154.69	49.85%
	7	27.0	287.42	208.23	141.74	49.31%
		26.2	287.99	185.93	117.01	40.63%
		27.5	195.06	140.74	85.28	43.72%
		24.6	273.08	181.86	109.16	39.97%
		25.5	274.23	184.41	147.32	53.72%
	14	25.0	226.90	141.39	121.52	53.56%
		26.5	215.38	117.49	76.13	35.35%
		23.2	235.63	161.53	142.08	60.30%
		26.4	217.38	161.75	137.18	63.11%
		27.9	217.47	165.34	79.02	36.34%
	28	22.7	195.90	150.79	97.98	50.02%
		24.1	195.09	122.77	74.13	38.00%
		28.3	158.27	76.35	49.42	31.23%
		27.5	257.99	183.18	100.37	38.90%
		25.2	102.92	83.94	44.68	43.41%
	35	30.7	177.81	119.61	72.96	41.03%
		24.7	172.68	89.14	54.38	31.49%
		25.1	196.36	118.66	73.81	37.59%
		22.3	199.71	96.47	68.19	34.14%
Medium sediment	0	26.5	1403.80	1014.78	852.45	60.72%
		30.0	978.60	607.75	447.39	45.72%
		28.8	846.05	662.58	511.23	60.43%
		27.4	1232.05	926.21	774.66	62.88%
		27.0	872.74	575.49	472.69	54.16%
	7	27.0	1056.19	923.47	782.54	74.09%
		27.1	861.43	634.10	503.73	58.48%
		25.0	1025.93	661.90	566.42	55.21%
		24.6	1147.82	783.21	679.08	59.16%
		27.5	969.81	743.44	592.93	61.14%
	14	26.8	764.35	545.17	456.40	59.71%
		29.9	781.51	561.87	407.55	52.15%
		24.8	1135.56	815.32	742.09	65.35%
		28.0	894.88	632.21	519.12	58.01%
		27.4	876.66	590.57	496.65	56.65%
	28	24.0	887.89	630.02	481.60	54.24%
		27.2	783.82	550.50	419.92	53.57%
		24.0	896.24	560.97	432.27	48.23%
		26.2	799.50	502.60	380.65	47.61%
		29.8	975.73	680.50	567.02	58.11%
	35	28.5	553.67	393.35	316.63	57.19%
		27.4	776.97	566.92	469.09	60.37%
		29.3	1070.54	862.09	729.31	68.13%
		26.6	624.15	458.48	376.35	60.30%
		24.3	632.08	504.47	438.06	69.30%
		25.4	520.03	401.59	287.90	55.36%

Continued

Appendix 3 (continued) Butyltin concentrations in whole tissues of *Hinia reticulata* during depuration after uptake from sediments and water.

Exposure regime	Day	Size (mm)	'Total' butyltins (ng g^{-1})	'TBT + DBT' (ng g^{-1})	'TBT' (ng g^{-1})	%TBT
High water	0	27.9	2607	1816	1199	45.99%
		26.4	2331	1511	909	38.98%
		28.3	2045	1320	861	42.10%
		27.6	2294	1625	1051	45.81%
		27.7	2414	1590	979	40.55%
	7	28.5	1544	1006	648	41.96%
		28.8	1777	1163	718	40.40%
		26.5	1555	988	650	41.80%
		25.8	2083	1535	857	41.17%
		24.3	2713	2080	1522	56.09%
	14	26.4	1696	1131	737	43.48%
		28.1	1699	1167	704	41.45%
		23.5	1890	1144	820	43.39%
		26.3	1766	1160	819	46.38%
		28.2	1538	1051	673	43.73%
	28	28.2	1611	1181	796	49.40%
		25.5	1791	1162	624	34.83%
		25.5	1394	977	743	53.28%
		26.0	1686	1070	737	43.72%
		27.5	1332	754	469	35.20%
	35	28.2	1151	706	415	36.04%
		26.7	1201	765	493	41.02%
		26.8	1230	853	486	39.49%
		27.7	1345	803	472	35.13%
		24.9	1529	929	653	42.69%
		24.6	984	558	310	31.46%
High sediment	0	26.2	14065	14530	11524	81.93%
		27.7	16305	13373	10607	65.05%
		28.4	16653	12732	9998	60.04%
		28.9	12477	8894	6189	49.61%
		28.0	18491	15451	12210	66.03%
	7	29.2	14863	11199	8633	58.08%
		23.7	18533	14840	11962	64.54%
		25.2	16823	12117	9960	59.20%
		28.7	14614	10299	7542	51.61%
		26.2	14167	11747	9365	66.10%
	14	27.3	14518	10265	7071	48.71%
		24.7	10943	7494	5511	50.36%
		26.2	17163	13166	10431	60.77%
		27.2	11973	8408	5717	47.75%
		25.9	12817	8814	6906	53.88%
	28	30.4	13816	11485	8826	63.89%
		24.3	9480	8113	5700	60.13%
		24.3	12354	11863	10069	81.50%
		25.9	10377	8633	7051	67.94%
		28.2	14029	9644	7406	52.79%
	35	26.8	9965	7044	5463	54.82%
		27.6	11350	7410	4565	40.22%
		25.4	11766	8282	6130	52.09%
		27.7	7540	7247	5825	77.25%
		24.7	11761	8577	6200	52.72%
		24.4	6736	4529	3859	57.28%

Appendix 4 Tissue concentrations and body burdens of butyltins in *Hinia reticulata* during depuration after injection of TBT.

Day		Shell length (mm)	Tissue dry weight (g)	Tissue Concentrations				Body Burdens		
				Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total (ng)	TBT+DBT (ng)	TBT (ng)
0	mean	27.28	0.1947	857.5	698.9	630.6	74.1%	153.44	126.76	113.71
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.65	0.0552	316.6	238.0	228.9	10.8%	28.90	24.44	22.97
	max.	29.50	0.2852	1293.9	1011.5	919.6	83.4%	207.19	142.52	128.20
	min.	24.30	0.1298	466.7	302.8	252.0	54.0%	90.94	59.00	49.11
1	mean	26.81	0.1758	835.7	719.4	661.2	79.0%	142.18	121.83	111.97
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.81	0.0347	167.6	155.7	143.4	4.5%	12.72	9.48	8.50
	max.	29.40	0.2356	1094.2	930.7	846.1	85.9%	164.87	137.03	125.83
	min.	23.80	0.1304	598.0	488.1	450.2	71.2%	121.22	106.18	100.47
2	mean	27.20	0.1797	819.8	695.7	613.5	74.2%	137.32	115.24	101.59
	n	10	10	10	10	10	10	10	10	10
	std. dev.	2.42	0.0577	211.4	208.6	182.5	13.5%	10.42	22.89	19.45
	max.	31.40	0.2989	1178.5	928.9	831.4	99.3%	152.23	163.28	142.66
	min.	24.30	0.1242	509.2	245.0	223.5	43.9%	116.03	73.23	66.82
3	mean	27.36	0.1883	697.2	575.4	524.6	75.7%	126.93	104.72	95.15
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.65	0.0390	155.2	123.5	124.8	8.5%	19.16	11.82	10.56
	max.	30.20	0.2281	1018.4	877.3	840.0	97.6%	156.98	122.43	111.91
	min.	24.50	0.1100	443.6	462.2	418.6	69.0%	96.89	78.89	71.27
4	mean	26.87	0.1826	641.4	519.4	457.5	71.8%	108.64	87.71	77.49
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.77	0.0406	257.3	214.4	183.6	4.1%	28.96	22.41	19.93
	max.	29.00	0.2303	1149.3	964.2	835.3	76.6%	130.02	108.77	93.13
	min.	23.90	0.0999	126.6	117.8	96.6	66.0%	29.15	27.12	22.24
9	mean	26.03	0.1321	760.4	608.4	530.9	70.1%	94.00	75.46	65.79
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.58	0.0311	241.3	187.7	164.1	2.5%	11.38	9.02	7.65
	max.	28.40	0.1827	1074.8	884.1	763.4	74.7%	107.38	89.27	77.07
	min.	23.70	0.0895	461.3	399.8	328.7	67.5%	71.31	59.34	53.24
14	mean	26.24	0.1537	501.7	402.2	349.7	69.2%	73.94	59.68	51.73
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.57	0.0352	125.6	105.3	101.4	7.2%	14.52	14.61	13.93
	max.	28.90	0.2389	628.0	509.4	462.5	84.4%	90.88	86.82	76.00
	min.	24.50	0.1210	200.7	165.6	131.9	59.3%	47.96	39.57	31.51
21	mean	26.14	0.1250	564.9	453.5	371.2	64.7%	68.30	54.29	44.30
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.28	0.0249	110.0	115.8	102.2	6.2%	5.00	6.18	6.00
	max.	29.10	0.1662	717.6	610.8	515.9	71.9%	72.79	60.91	51.44
	min.	24.80	0.0930	415.8	279.7	228.7	53.8%	59.95	40.32	33.67

Continued

Appendix 4 (continued) Tissue concentrations and body burdens of butyltins in *Hinia reticulata* during depuration after injection of TBT.

Day		Shell length (mm)	Tissue dry weight (g)	Tissue Concentrations				Body Burdens		
				Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total (ng)	TBT+DBT (ng)	TBT (ng)
28	mean	26.25	0.1220	313.8	248.7	201.8	65.0%	38.46	30.62	24.96
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.55	0.0250	114.9	98.6	74.6	9.9%	16.62	14.24	11.57
	max.	28.60	0.1535	448.9	374.0	294.6	73.9%	62.70	50.26	42.45
	min.	23.70	0.0862	154.4	113.9	91.5	45.1%	14.14	11.06	10.06
35	mean	25.98	0.1209	362.3	262.3	216.6	60.4%	43.18	31.45	25.82
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.94	0.0297	87.7	63.2	51.4	6.7%	12.38	10.06	7.64
	max.	30.60	0.1707	438.7	350.4	291.3	73.0%	66.61	52.19	40.67
	min.	24.00	0.0919	170.7	140.8	124.5	52.9%	17.40	14.35	12.69
42	mean	25.99	0.1025	377.3	309.0	193.9	52.5%	37.47	30.72	19.71
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.54	0.0228	100.2	74.7	56.6	10.6%	10.58	7.86	7.30
	max.	28.70	0.1359	468.3	387.2	278.4	68.7%	58.24	43.40	32.65
	min.	23.20	0.0633	150.3	129.5	103.2	35.8%	20.42	17.59	12.06
56	mean	26.49	0.0970	291.8	232.6	122.6	42.3%	27.02	21.56	11.48
	n	10	10	10	10	10	10	10	10	10
	std. dev.	2.15	0.0241	82.3	63.8	34.1	5.7%	5.11	4.05	2.99
	max.	30.90	0.1420	425.5	331.5	176.7	51.9%	33.75	26.95	17.47
	min.	23.20	0.0733	160.3	124.3	61.2	34.8%	17.92	13.69	7.07
70	mean	26.51	0.0843	259.9	206.2	92.6	35.9%	21.04	16.62	7.66
	n	10	10	10	10	10	10	10	10	10
	std. dev.	1.62	0.0184	68.4	60.7	33.2	10.3%	3.17	2.81	2.56
	max.	29.40	0.1137	421.6	347.8	145.2	44.1%	24.94	20.58	10.71
	min.	24.00	0.0592	157.4	114.4	22.2	9.4%	16.74	13.01	1.58
91	mean	26.36	0.0781	178.2	147.5	46.4	26.4%	13.86	11.49	4.01
	n	10	10	10	10	10	10	10	10	10
	std. dev.	2.24	0.0227	35.0	28.2	23.1	12.4%	4.73	4.00	2.92
	max.	30.50	0.1081	236.9	191.8	93.5	43.8%	22.57	18.70	9.89
	min.	23.60	0.0491	130.1	112.2	16.2	11.3%	7.87	7.10	1.02

Appendix 5 Butyltin concentrations in *Hinia reticulata* with normal or ligatured proboscis, exposed to TBT in sediments and/or water.

Exposure route	Day	Ligatured/Normal	Size (mm)	Dry weight (g)	Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
Sediment	7	Ligatured	29.3	0.1541	2086.1	1774.6	1605.7	77.0%
	7	Ligatured	29.1	0.1371	1976.0	1450.4	1336.0	67.6%
	7	Ligatured	28.3	0.2248	1084.0	901.0	772.4	71.3%
	7	Ligatured	28.4	0.1212	1906.2	1434.8	1309.8	68.7%
	7	Ligatured	27.9	0.1954	1516.3	1212.7	1127.4	74.4%
	7	Ligatured	28.4	0.1072	1414.4	1133.3	997.8	70.5%
	7	Ligatured	28.1	0.1504	1596.8	1245.2	1147.5	71.9%
	7	Ligatured	26.7	0.0911	2122.3	1824.4	1707.2	80.4%
	7	Ligatured	26.4	0.1513	1624.8	1389.4	1259.7	77.5%
	7	Normal	27.2	0.1301	2320.0	2135.6	1895.9	81.7%
	7	Normal	29.8	0.1772	1979.2	1571.5	1367.1	69.1%
	7	Normal	29.2	0.2076	1917.4	1652.5	1396.4	72.8%
	7	Normal	27.4	0.1982	1239.7	1107.9	1004.5	81.0%
	7	Normal	27.7	0.2233	1585.8	1254.9	1087.7	68.6%
	7	Normal	27.5	0.1313	1536.3	1071.3	989.8	64.4%
	7	Normal	28.4	0.2045	1880.5	1589.0	1421.9	75.6%
	7	Normal	29.6	0.1586	2081.5	1549.2	1427.8	68.6%
	7	Normal	27.2	0.1207	1900.6	1565.6	1436.8	75.6%
	7	Normal	29.9	0.1909	1589.1	1322.7	1191.5	75.0%
Water	7	Ligatured	25.4	0.0997	197.5	146.6	97.6	49.4%
	7	Ligatured	28.2	0.1560	103.4	73.7	49.1	47.5%
	7	Ligatured	29.7	0.2282	123.3	90.4	67.7	54.9%
	7	Ligatured	23.5	0.1167	155.0	115.1	81.0	52.3%
	7	Ligatured	27.5	0.1028	242.1	196.6	156.6	64.7%
	7	Ligatured	28.2	0.1318	141.6	107.0	66.0	46.6%
	7	Ligatured	27.8	0.1743	154.8	99.1	77.9	50.3%
	7	Ligatured	26.4	0.1001	178.9	162.9	125.7	70.3%
	7	Ligatured	29.2	0.2210	140.2	97.4	70.1	50.0%
	7	Normal	29.7	0.1415	203.8	151.4	118.4	58.1%
	7	Normal	29.3	0.1685	135.3	92.0	66.2	48.9%
	7	Normal	29.5	0.1942	139.3	91.9	58.6	42.0%
	7	Normal	27.7	0.1726	111.4	75.1	52.2	46.9%
	7	Normal	31.5	0.2632	118.2	86.0	56.2	47.6%
	7	Normal	28.4	0.1529	165.6	127.0	86.8	52.4%
	7	Normal	28.2	0.2491	104.4	57.1	42.2	40.5%
	7	Normal	28.7	0.2556	109.1	82.9	58.1	53.2%
	7	Normal	27.4	0.1545	152.7	109.5	98.8	64.7%
	7	Normal	28.3	0.1580	158.9	112.9	70.9	44.6%

Continued

Appendix 5 (continued) Butyltin concentrations in *Hinia reticulata* with normal or ligatured proboscis, exposed to TBT in sediments and/or water.

Exposure route	Day	Ligatured/Normal	Size (mm)	Dry weight (g)	Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
Sediment	15	Ligatured	30.4	0.2100	2076.9	1536.2	1189.3	57.3%
	15	Ligatured	28.8	0.1614	2747.9	2403.7	1894.9	69.0%
	15	Ligatured	28.9	0.1538	3569.8	2710.7	2329.9	65.3%
	15	Ligatured	26.2	0.0852	3693.3	3004.1	2723.9	73.8%
	15	Ligatured	27.0	0.1081	3415.1	2729.5	2374.5	69.5%
	15	Ligatured	26.2	0.1211	3023.0	2309.5	2133.6	70.6%
	15	Ligatured	27.3	0.1201	3277.0	2361.1	2034.6	62.1%
	15	Ligatured	27.1	0.1197	2904.2	2198.7	1946.9	67.0%
	15	Ligatured	27.9	0.1813	2126.8	1629.2	1464.5	68.9%
	15	Ligatured	26.3	0.1025	3142.6	2475.1	2178.1	69.3%
	15	Normal	28.2	0.1581	3346.6	2974.3	2528.0	75.5%
	15	Normal	27.7	0.1207	3538.6	2934.6	2510.0	70.9%
	15	Normal	27.8	0.1459	3202.4	2698.5	2290.7	71.5%
	15	Normal	28.7	0.1579	3751.8	3165.2	2740.5	73.0%
	15	Normal	27.5	0.1891	2625.6	1907.8	1483.8	56.5%
	15	Normal	27.6	0.1581	3847.1	3041.6	2694.9	70.0%
	15	Normal	29.2	0.1747	3427.7	2770.2	2689.7	78.5%
	15	Normal	28.8	0.1687	2879.0	2149.4	1660.0	57.7%
	15	Normal	27.5	0.1554	3954.0	3142.8	2931.0	74.1%
Water	15	Ligatured	29.1	0.2326	278.6	176.7	117.8	42.3%
	15	Ligatured	26.4	0.0666	473.7	380.3	295.4	62.4%
	15	Ligatured	27.8	0.1536	304.5	217.4	150.7	49.5%
	15	Ligatured	27.7	0.1343	271.6	208.0	150.6	55.5%
	15	Ligatured	28.2	0.1394	263.7	198.7	137.5	52.1%
	15	Ligatured	24.6	0.0914	286.3	207.7	125.5	43.8%
	15	Ligatured	27.2	0.2248	251.8	185.7	142.0	56.4%
	15	Ligatured	26.1	0.0932	356.1	256.3	185.0	52.0%
	15	Ligatured	29.3	0.1757	406.9	312.9	244.1	60.0%
	15	Normal	29.2	0.1318	434.1	332.1	227.0	52.3%
	15	Normal	30.9	0.1420	314.3	245.2	150.8	48.0%
	15	Normal	28.7	0.1876	312.0	239.9	160.6	51.5%
	15	Normal	27.9	0.1391	364.2	305.6	202.6	55.6%
	15	Normal	28.6	0.1994	286.2	205.2	135.2	47.2%
	15	Normal	28.3	0.1516	515.5	380.3	276.4	53.6%
	15	Normal	29.4	0.1972	313.3	237.3	172.0	54.9%
	15	Normal	30.2	0.2649	230.7	186.3	119.0	51.6%
	15	Normal	29.2	0.2000	309.5	226.4	139.4	45.0%
	15	Normal	28.3	0.1303	359.5	275.4	197.9	55.1%

Appendix 6 Butyltin concentrations in tissues of *Hinia reticulata* during uptake and depuration when exposed to TBT contaminated sediment and water.

(All values are means (*n=10*) and concentration units are in ng g^{-1} dry weight)

			Gill				Kidney				Digestive Gland				Head/Foot				Remaining Tissue			
	Day	Exposure	Tot.	T + D	TBT	% TBT	Tot.	T + D	TBT	% TBT	Tot.	T + D	TBT	% TBT	Tot.	T + D	TBT	% TBT	Tot.	T + D	TBT	% TBT
Uptake	5	Sediment	5869	5578	4422	*75.3%*	3343	2646	1955	*58.5%*	854	524	356	*41.6%*	2668	2654	2510	*94.1%*	1444	1334	1295	*89.7%*
	12	Sediment	12704	11870	10337	*81.4%*	6989	5298	5348	*76.5%*	1076	851	583	*54.2%*	7402	6624	6239	*84.3%*	2613	2398	1940	*74.3%*
	19	Sediment	14684	13790	14030	*95.5%*	9711	7575	7817	*80.5%*	2697	1577	1845	*68.4%*	9251	8790	9628	*104.1%*	3949	3560	2880	*72.9%*
	26	Sediment	17201	15619	13353	*77.6%*	14731	11662	8349	*56.7%*	2376	1577	988	*41.6%*	11388	10686	9694	*85.1%*	5226	4645	3789	*72.5%*
	33	Sediment	17243	15568	13431	*77.9%*	16749	13530	9222	*55.1%*	3246	2258	1207	*37.2%*	11886	10974	11447	*96.3%*	5882	5138	5382	*91.5%*
	40	Sediment	21385	17461	14600	*68.3%*	18582	14303	9647	*51.9%*	5281	3276	1962	*37.2%*	12831	10358	9905	*77.2%*	6769	5948	5183	*76.6%*
Depuration	47	Sediment	13646	11341	8517	*62.4%*	16475	12918	8979	*54.5%*	5550	3642	1871	*33.7%*	10210	7530	6924	*67.8%*	5661	5204	3991	*70.5%*
	54	Sediment	12022	9194	6461	*53.7%*	13373	10748	7517	*56.2%*	5517	4101	2281	*41.3%*	8965	7560	7892	*88.0%*	5985	4934	3830	*64.0%*
	61	Sediment	11416	7451	5545	*48.6%*	12683	9795	7491	*59.1%*	6018	3822	1379	*22.9%*	9063	6270	5837	*64.4%*	6984	5493	4498	*64.4%*
	75	Sediment	7959	5072	3842	*48.3%*	7969	5899	4363	*54.8%*	5213	3016	1120	*21.5%*	7043	5193	4722	*67.1%*	5511	4437	3706	*67.3%*
	89	Sediment	6361	3687	2405	*37.8%*	7026	4916	3119	*44.4%*	4476	2774	1114	*24.9%*	4801	3586	3136	*65.3%*	4610	3773	2231	*48.4%*
	103	Sediment	5549	3292	2102	*37.9%*	5891	4655	2914	*49.5%*	5144	3291	980	*19.1%*	4189	2964	2628	*62.7%*	4162	3288	2493	*59.9%*
Uptake	5	Water	1050	1025	836	*79.6%*	346	264	248	*71.8%*	57	41	26	*46.4%*	194	159	177	*91.1%*	160	138	147	*91.3%*
	12	Water	2294	2036	1529	*66.6%*	1139	688	387	*33.9%*	171	114	72	*41.9%*	419	355	370	*88.2%*	327	290	290	*88.6%*
	19	Water	1825	1587	1155	*63.3%*	1216	822	473	*38.9%*	243	181	107	*44.0%*	543	412	450	*82.8%*	400	334	328	*82.1%*
	26	Water	1913	1511	1050	*54.9%*	1524	931	475	*31.1%*	374	247	144	*38.6%*	524	461	372	*71.0%*	416	354	268	*64.3%*
	33	Water	2048	1602	1158	*56.5%*	1717	1125	543	*31.6%*	425	269	140	*32.8%*	565	471	400	*70.7%*	505	415	319	*63.3%*
	40	Water	2210	1655	1025	*46.4%*	1804	1093	439	*24.3%*	611	395	199	*32.5%*	650	496	386	*59.4%*	547	429	281	*51.4%*
Depuration	47	Water	1683	1177	723	*42.9%*	1116	772	390	*35.0%*	684	475	177	*25.8%*	473	347	257	*54.2%*	504	414	250	*49.6%*
	54	Water	1521	914	491	*32.3%*	1038	695	349	*33.6%*	749	475	156	*20.8%*	357	300	271	*75.8%*	429	349	217	*50.7%*
	61	Water	1328	787	479	*36.1%*	716	510	274	*38.3%*	569	332	119	*20.9%*	381	283	239	*62.8%*	417	320	218	*52.3%*
	75	Water	1122	627	414	*36.9%*	755	381	236	*31.3%*	475	288	53	*11.2%*	314	244	98	*31.1%*	399	283	189	*47.5%*
	89	Water	1092	640	400	*36.6%*	454	287	188	*41.4%*	484	292	92	*19.1%*	259	177	199	*76.9%*	349	260	168	*48.0%*
	103	Water	806	354	159	*19.8%*	423	300	159	*37.5%*	609	368	87	*14.3%*	236	153	117	*49.4%*	321	240	116	*36.1%*

Tot. = 'Total butyltins' (optisolv extractable); T + D = hexane extractable 'TBT+DBT'; TBT = NaOH washed hexane extractable 'TBT'

Appendix 7. Butyltin burdens in tissues of *Hinia reticulata* during uptake and depuration when exposed to TBT contaminated sediment and water.

(All values are means (*n=10*) and units are ng)

	Day	Exposure	Gill			Kidney			Digestive Gland			Head/Foot			Remaining Tissue		
			Total	TBT+DBT	TBT	Total	TBT+DBT	TBT	Total	TBT+DBT	TBT	Total	TBT+DBT	TBT	Total	TBT+DBT	TBT
Uptake	5	Sediment	49.5	47.1	37.3	14.4	11.4	8.4	52.5	32.2	21.9	191.1	190.1	179.8	84.1	77.7	75.4
	12	Sediment	95.4	89.1	77.6	35.6	27.0	27.3	80.0	63.3	43.3	524.3	469.3	442.0	169.5	155.5	125.9
	19	Sediment	96.7	90.8	92.4	40.5	31.6	32.6	119.1	69.6	81.5	585.9	556.7	609.8	210.7	189.9	153.7
	26	Sediment	118.8	107.9	92.3	59.1	46.8	33.5	164.5	109.2	68.4	745.8	699.9	634.9	301.9	268.4	218.9
	33	Sediment	124.5	112.4	97.0	70.3	56.8	38.7	215.9	150.2	80.3	791.1	730.4	761.9	347.1	303.2	317.6
	40	Sediment	122.5	100.0	83.6	66.1	50.9	34.3	212.9	132.1	79.1	759.2	612.9	586.1	338.0	297.0	258.8
Depuration	47	Sediment	80.5	66.9	50.2	62.6	49.1	34.1	268.0	175.9	90.3	657.4	484.8	445.8	303.6	279.1	214.0
	54	Sediment	73.1	55.9	39.3	51.6	41.5	29.0	226.0	168.0	93.4	561.6	473.6	494.4	333.9	275.3	213.7
	61	Sediment	68.7	44.8	33.4	49.9	38.6	29.5	265.3	168.5	60.8	535.3	370.3	344.7	339.9	267.3	218.9
	75	Sediment	42.9	27.4	20.7	27.8	20.6	15.2	185.6	107.4	39.9	364.2	268.6	244.2	237.7	191.4	159.9
	89	Sediment	35.7	20.7	13.5	23.6	16.5	10.5	143.1	88.7	35.6	225.7	168.6	147.5	185.4	151.7	89.7
	103	Sediment	30.3	18.0	11.5	18.5	14.6	9.2	166.7	106.7	31.8	211.6	149.8	132.7	170.2	134.5	102.0
Uptake	5	Water	8.1	7.9	6.5	1.5	1.1	1.1	5.1	3.7	2.4	14.0	11.4	12.8	10.1	8.7	9.2
	12	Water	16.6	14.7	11.0	5.2	3.1	1.8	12.5	8.3	5.3	27.8	23.6	24.5	22.8	20.2	20.2
	19	Water	13.4	11.7	8.5	5.4	3.7	2.1	19.9	14.8	8.8	38.6	29.3	32.0	24.5	20.4	20.1
	26	Water	12.0	9.5	6.6	6.1	3.7	1.9	19.6	13.0	7.6	33.4	29.4	23.7	22.9	19.5	14.7
	33	Water	14.4	11.2	8.1	7.6	5.0	2.4	24.9	15.7	8.2	40.2	33.5	28.4	30.5	25.1	19.3
	40	Water	13.9	10.4	6.5	6.7	4.0	1.6	29.8	19.3	9.7	38.5	29.4	22.9	26.6	20.9	13.7
Depuration	47	Water	9.3	6.5	4.0	4.3	3.0	1.5	31.4	21.8	8.1	28.3	20.8	15.4	25.1	20.6	12.4
	54	Water	8.5	5.1	2.7	3.7	2.5	1.2	21.5	13.6	4.5	19.8	16.6	15.0	20.0	16.3	10.1
	61	Water	7.6	4.5	2.7	2.6	1.9	1.0	21.4	12.5	4.5	20.4	15.2	12.8	19.3	14.8	10.1
	75	Water	6.4	3.6	2.4	2.6	1.3	0.8	15.6	9.5	1.8	15.6	12.1	4.8	16.4	11.6	7.8
	89	Water	5.6	3.3	2.0	1.4	0.9	0.6	12.1	7.3	2.3	11.7	8.0	9.0	12.6	9.4	6.1
	103	Water	4.1	1.8	0.8	1.3	0.9	0.5	13.5	8.1	1.9	10.7	7.0	5.3	12.3	9.2	4.5

Appendix 8 TBT concentrations in water and sediments – Talland Bay sediment

Exposure route	Day	Water			Sediment		
		TBT + DBT (ng l^{-1})	TBT (ng l^{-1})	% TBT	TBT + DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
Sediment	-7				900.9	882.5	98.0%
	0	346.89	255.23	73.58%			
	2	980.03	824.55	84.14%			
	4	943.52	750.64	79.56%			
	7	892.79	661.81	74.13%			
	10	1376.03	1074.77	78.11%			
	13	528.96	364.07	68.83%			
	16	898.72	664.43	73.93%			
	21	286.21	178.69	62.43%			
	24	237.25	153.14	64.55%			
	28	184.07	112.62	61.19%			
	30				264.1	252.0	95.4%
	36	149.33	144.33	96.65%			
	50	64.17	45.02	70.16%			
	59	76.98	52.57	68.29%			
	60				224.6	193.3	86.1%
	67	40.59	15.77	38.85%			
	78	58.31	30.06	51.56%			
	91	63.89	16.87	26.40%			
	112	10.03	2.86	28.53%	77.9	62.3	79.7%
	123	91.45	59.57	65.14%			
	136	59.98	29.55	49.27%			
	151	18.86	4.53	24.00%			
Water	0	1659.27	1518.56	91.52%			
	2						
	4						
	7	586.02	462.22	78.88%			
	10	328.06	233.18	71.08%			
	13	817.36	665.87	81.47%			
	16	366.67	214.18	58.41%			
	21	273.93	161.03	58.79%			
	24	177.40	77.34	43.60%			
	28	135.40	59.12	43.66%			
	30						
	36	127.66	62.31	48.81%			
	50	84.99	44.56	52.43%			
	59	87.41	46.03	52.66%			
	60						
	67	91.70	44.68	48.73%			
	78	72.77	42.85	58.88%			
	91	66.61	49.46	74.25%			
	112	27.52	17.35	63.06%			
	123	54.59	27.04	49.53%			
	136	39.76	16.74	42.11%			
	151	38.31	22.55	58.86%			

Appendix 9 TBT concentrations in water and sediments – East Looe sediment

Exposure route	Day	Water			Sediment		
		TBT + DBT ($ng\ l^{-1}$)	TBT ($ng\ l^{-1}$)	% TBT	TBT + DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
Sediment	-7				1228.7	1192.9	97.1%
	0	10.56	10.15	96.15%			
	2	78.86	72.05	91.36%			
	4	42.75	35.59	83.24%			
	7	51.82	44.93	86.71%			
	10	67.22	63.25	94.09%			
	13	60.26	53.71	89.13%			
	16	75.11	61.09	81.35%			
	21	70.27	60.93	86.70%			
	24	47.81	41.89	87.62%			
	28	50.46	43.73	86.67%			
	30				1019.3	984.2	96.6%
	36	43.40	39.87	91.86%			
	50	44.91	39.29	87.50%			
	59	45.42	40.21	88.54%			
	60				1136.7	1048.7	92.3%
	67	19.24	17.68	91.90%			
	78	30.22	24.72	81.82%			
	91	32.02	22.06	68.87%			
	112	12.15	8.85	72.85%	775.1	710.1	91.6%
	123	36.69	30.21	82.35%			
	136	37.61	26.90	71.51%			
	151	27.38	22.84	83.45%			
Water	0	97.73	97.74	100.01%			
	2	31.87	29.13	91.39%			
	4	91.78		0.00%			
	7	44.25	35.84	81.01%			
	10	20.66	14.52	70.26%			
	13	23.79	17.42	73.21%			
	16	17.86	11.11	62.21%			
	21	24.35	17.88	73.43%			
	24	18.23	12.61	69.17%			
	28	20.32	15.25	75.05%			
	30						
	36	31.54	24.24	76.87%			
	50	22.37	16.06	71.80%			
	59	19.83	12.63	63.68%			
	60						
	67	14.96	9.96	66.53%			
	78	14.00	9.78	69.87%			
	91	11.21	5.91	52.72%			
	112	16.93	12.49	73.76%			
	123	12.16	7.08	58.26%			
	136	13.18	6.54	49.63%			
	151	12.27	8.18	66.69%			

Appendix 10 TBT concentrations in water and sediments – St. John's Lake sediment

Exposure route	Day	Water			Sediment		
		TBT + DBT (ng l^{-1})	TBT (ng l^{-1})	% TBT	TBT + DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
Sediment	-7				1195.6	1171.9	98.0%
	0	10.90	5.66	51.92%			
	2	63.74	59.28	93.00%			
	4	29.24	22.53	77.04%			
	7	34.06	29.14	85.56%			
	10	35.57	30.91	86.91%			
	13	27.45	23.39	85.21%			
	16	33.02	25.91	78.47%			
	21	22.94	17.44	76.05%			
	24	11.86	9.18	77.40%			
	28	11.93	8.81	73.85%			
	30				1060.6	1030.4	97.2%
	36	25.46	22.28	87.51%			
	50	11.11	8.35	75.21%			
	59	10.28	9.25	89.93%			
	60				1080.3	988.3	91.5%
	67	11.44	9.88	86.34%			
	78	16.80	11.99	71.34%			
	91	23.54	17.48	74.25%			
	112	4.22	3.09	73.25%	867.1	804.5	92.8%
	123	24.68	20.76	84.14%			
	136	20.99	15.50	73.87%			
	151	21.01	15.96	75.97%			
Water	0	57.80	49.30	85.29%			
	2	25.21	26.71	105.95%			
	4	69.66	72.31	103.80%			
	7	34.20	30.58	89.41%			
	10	16.73	12.43	74.30%			
	13	16.76	12.32	73.51%			
	16	13.22	7.44	56.30%			
	21	56.77	48.83	86.02%			
	24	36.03	28.20	78.29%			
	28	35.66	29.23	81.97%			
	30						
	36	25.87	19.06	73.69%			
	50	38.06	30.35	79.75%			
	59	38.94	25.50	65.48%			
	60						
	67	19.76	12.55	63.52%			
	78	11.26	8.04	71.42%			
	91	5.89	3.10	52.57%			
	112	5.15	3.73	72.46%			
	123	9.56	6.55	68.46%			
	136	11.82	6.94	58.68%			
	151	7.85	3.45	43.87%			

Appendix 11 Butyltin concentrations in *Hinia reticulata* exposed to TBT in Talland Bay sediments and overlying water

Day	Water-only exposed				Sediment and water exposed			
	Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT	Total (ng g^{-1})	TBT+DBT (ng g^{-1})	TBT (ng g^{-1})	% TBT
10	2827.0	2036.6	1695.6	60.0%	8700.3	7661.9	6298.0	72.4%
10	2417.4	1867.1	1506.0	62.3%	11123.8	8212.2	6912.9	62.1%
10	2643.8	2013.2	1686.4	63.8%	13206.6	9832.2	7560.5	57.2%
10	2801.3	1927.4	1516.4	54.1%	13933.6	10183.8	7892.0	56.6%
10	3054.2	2271.6	1883.6	61.7%	14102.9	12185.9	9990.7	70.8%
10	1858.0	1459.3	1095.9	59.0%	12165.7	9423.4	7597.3	62.4%
10	2864.4	2202.2	1765.6	61.6%	18306.5	12869.5	10415.9	56.9%
10	3568.2	2802.9	2282.6	64.0%	9023.3	6502.9	5250.6	58.2%
10	3363.0	2686.1	2163.3	64.3%	14796.9	12746.0	10185.4	68.8%
10	5494.3	3571.2	2771.0	50.4%	8589.2	6420.2	5273.0	61.4%
30	4582.1	3835.5	3208.1	70.0%	8002.3	6429.1	4663.0	58.3%
30	3000.1	2437.1	1892.3	63.1%	12060.0	10272.2	7424.2	61.6%
30	4211.5	3295.6	2329.0	55.3%	15855.5	13521.3	10216.9	64.4%
30	4304.7	3458.3	2442.6	56.7%	10649.5	7947.8	6152.8	57.8%
30	2709.3	2076.6	1559.4	57.6%	9865.4	7580.8	5570.3	56.5%
30	3136.1	2231.4	1517.7	48.4%	10215.9	7535.1	5695.8	55.8%
30	4064.0	2931.0	2099.4	51.7%	12325.2	10620.7	8045.3	65.3%
30	3170.2	2456.7	1706.3	53.8%	11517.4	8931.9	6457.3	56.1%
30	3074.2	2166.4	1409.5	45.9%	11530.1	8487.7	5392.3	46.8%
30	4443.2	3690.7	2900.0	65.3%	15717.7	13066.2	9488.5	60.4%
60	2693.3	1768.1	1307.3	48.5%				
60	3735.0	2301.3	1713.1	45.9%				
60	2417.8	1581.9	1049.4	43.4%				
60	2384.2	1550.8	1084.7	45.5%				
60	2191.0	1332.9	864.9	39.5%				
60	2843.5	1788.1	1349.2	47.4%				
60	3707.7	2192.8	1626.9	43.9%				
60	2626.3	1615.9	1142.4	43.5%				
60	3384.9	2083.7	1338.1	39.5%				
60	2989.5	1985.0	1388.7	46.5%				

Appendix 12 Butyltin concentrations in *Hinia reticulata* exposed to TBT in East Looe sediments and overlying water

Day	Water-only exposed				Sediment and water exposed			
	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
10	225.9	166.3	138.7	61.4%	635.4	490.2	452.1	71.2%
10	214.2	134.3	108.3	50.6%	574.2	414.9	368.3	64.1%
10	226.5	165.6	117.9	52.1%	492.1	409.8	362.8	73.7%
10	267.7	171.8	137.3	51.3%	524.7	383.7	344.8	65.7%
10	315.9	226.9	181.2	57.4%	738.1	586.8	521.9	70.7%
10	280.0	190.9	170.5	60.9%	779.4	627.2	590.8	75.8%
10	137.1	100.3	77.3	56.4%	842.9	656.0	644.8	76.5%
10	216.2	137.1	102.9	47.6%	539.7	442.1	405.8	75.2%
10	238.6	207.6	163.9	68.7%	731.4	664.2	598.3	81.8%
10	241.4	165.8	152.5	63.2%	622.3	506.9	473.5	76.1%
30	753.0	623.6	508.0	67.5%	943.8	692.3	573.6	60.8%
30	323.6	217.8	144.0	44.5%	1388.5	1095.4	890.1	64.1%
30	337.5	261.3	187.1	55.5%	845.7	757.4	716.4	84.7%
30	392.7	269.1	198.9	50.7%	1038.0	849.8	799.9	77.1%
30	481.0	355.3	261.4	54.4%	1818.4	1858.7	1687.1	92.8%
30	341.7	254.9	185.4	54.3%	1435.3	1120.9	985.8	68.7%
30	352.2	241.1	181.0	51.4%	1695.7	1328.5	1155.9	68.2%
30	336.3	250.0	183.1	54.4%	1549.0	1391.8	1245.8	80.4%
30	330.9	256.9	208.2	62.9%	1680.0	1216.2	1097.4	65.3%
30	400.2	271.5	233.3	58.3%	1033.7	814.4	745.8	72.1%
60	526.2	347.3	239.6	45.5%	1928.9	1423.8	1180.2	61.2%
60	496.8	302.8	197.1	39.7%	1424.1	999.9	877.9	61.6%
60	446.9	298.2	221.0	49.4%	1655.7	1342.5	1190.2	71.9%
60	568.8	405.2	308.7	54.3%	2086.7	1634.1	1395.1	66.9%
60	434.3	297.9	227.5	52.4%	1894.6	1479.4	1269.0	67.0%
60	413.6	284.5	206.7	50.0%	2075.8	1436.6	1213.8	58.5%
60	551.3	390.3	274.9	49.9%	1647.8	1256.1	1126.6	68.4%
60	377.4	264.5	198.0	52.5%	1239.7	883.9	764.4	61.7%
60	393.5	234.7	166.8	42.4%	1252.3	980.7	850.2	67.9%
60	382.4	258.8	175.1	45.8%	2319.3	1857.6	1565.4	67.5%

Appendix 13 Butyltin concentrations in *Hinia reticulata* exposed to TBT in St. John's Lake sediments and overlying water

Day	Water-only exposed				Sediment and water exposed			
	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT	Total ($ng\ g^{-1}$)	TBT+DBT ($ng\ g^{-1}$)	TBT ($ng\ g^{-1}$)	% TBT
10	137.9	107.4	88.5	64.2%	503.4	361.2	336.4	66.8%
10	219.1	154.5	125.2	57.2%	581.8	445.3	413.6	71.1%
10	260.4	189.2	198.5	76.2%	435.4	320.9	305.9	70.2%
10	328.3	260.9	220.8	67.2%	796.9	683.4	656.6	82.4%
10	233.8	186.5	150.2	64.3%	584.1	444.9	443.2	75.9%
10	249.3	186.8	158.0	63.4%	642.4	536.8	585.7	91.2%
10	242.9	195.1	179.2	73.8%	635.3	512.2	466.1	73.4%
10	199.1	132.6	115.5	58.0%	570.0	486.3	428.4	75.2%
10	175.0	135.4	113.3	64.8%	588.1	455.1	418.1	71.1%
10	174.1	127.8	108.0	62.0%	705.1	562.2	500.2	70.9%
30	385.9	308.9	222.2	57.6%	878.8	701.3	636.1	72.4%
30	622.7	476.2	373.2	59.9%	1154.2	959.9	850.0	73.6%
30	435.3	375.6	281.0	64.6%	1034.4	827.0	746.9	72.2%
30	562.3	518.2	349.6	62.2%	940.5	874.3	762.7	81.1%
30	531.3	425.4	308.7	58.1%	1342.9	1023.4	934.1	69.6%
30	375.7	280.6	205.3	54.6%	1308.3	1080.4	954.9	73.0%
30	570.4	651.6	328.0	57.5%	824.7	610.8	572.8	69.5%
30	561.9	437.8	333.1	59.3%	1057.4	860.2	741.2	70.1%
30	420.0	394.0	243.0	57.8%	893.1	697.2	633.2	70.9%
30	638.9	530.1	417.3	65.3%	973.7	724.0	655.0	67.3%
60	763.4	568.5	393.6	51.6%	2236.6	1612.4	1456.6	65.1%
60	1057.0	790.2	635.4	60.1%	815.0	636.7	569.8	69.9%
60	885.2	665.3	498.6	56.3%	1045.6	767.9	670.2	64.1%
60	788.2	742.7	621.8	78.9%	1221.7	998.0	898.4	73.5%
60	871.8	620.2	494.2	56.7%	1228.8	807.1	714.5	58.1%
60	667.1	509.9	394.1	59.1%	1248.0	877.9	779.6	62.5%
60	882.1	598.7	450.8	51.1%	1181.2	856.5	771.8	65.3%
60	821.3	579.4	404.7	49.3%	1458.4	1227.5	1125.4	77.2%
60	404.7	272.6	199.1	49.2%	1272.7	857.5	735.5	57.8%
60	791.3	505.6	400.9	50.7%	1017.5	703.0	632.5	62.2%

Appendix 14 Butyltin concentrations in tissues of *Hinia reticulata* during bioaccumulation from Talland Bay, East Looe and St. John's Lake sediments, and overlying waters.

(All values are from pooled tissues (*n=10*) and concentration units are in ng g^{-1} dry weight)

Sediment	Day	Exposure Route	Gill/Osphradium				Kidney				Digestive Gland				Head/Foot				Remaining tissue			
			Total	TBT +DBT	TBT	%TBT	Total	TBT +DBT	TBT	%TBT	Total	TBT +DBT	TBT	%TBT	Total	TBT +DBT	TBT	%TBT	Total	TBT +DBT	TBT	%TBT
Talland Bay	10	Sediment	40888.0	31575.9	24028.5	*59%*	27896.8	19176.5	11410.8	*41%*	4583.4	2573.1	1590.7	*35%*	10976.9	7531.5	6816.6	*62%*	7827.0	6396.9	5431.3	*69%*
	30		27146.3	20776.0	15083.0	*56%*	30166.2	22285.6	13187.8	*44%*	6501.9	4814.3	2456.1	*38%*	11241.0	9174.7	7545.2	*67%*	11081.7	8133.2	6965.3	*63%*
	60		*No survivors after 60 days*				*No survivors after 60 days*				*No survivors after 60 days*				*No survivors after 60 days*				*No survivors after 60 days*			
	10	Water	12828.4	9984.5	7691.2	*60%*	6633.3	4083.2	2577.4	*39%*	1070.8	768.7	471.7	*44%*	2445.7	1612.0	1343.4	*55%*	2459.7	1939.6	1548.3	*63%*
	30		10512.8	8314.0	5715.1	*54%*	8289.9	6409.1	4132.4	*50%*	2635.9	1893.1	1144.1	*43%*	3869.3	2467.8	2013.1	*52%*	4126.4	3127.1	2444.7	*59%*
	60		7098.6	4641.7	2736.1	*39%*	6405.5	4660.3	2272.6	*35%*	2505.0	1511.0	638.3	*25%*	2014.5	1585.8	1318.2	*65%*	2386.9	1862.1	1438.7	*60%*
East Looe	10	Sediment	2226.4	1778.1	1667.2	*75%*	835.7	566.2	508.1	*61%*	117.4	83.2	57.9	*49%*	894.6	630.7	644.9	*72%*	390.8	277.0	239.5	*61%*
	30		4108.4	3148.4	2749.7	*67%*	2032.4	1469.5	1096.6	*54%*	442.4	321.8	193.4	*44%*	1670.4	1336.1	1189.7	*71%*	770.1	644.5	580.2	*75%*
	60		3970.3	3113.2	2715.0	*68%*	2572.1	1669.0	1310.5	*51%*	594.5	385.3	183.7	*31%*	2053.0	1236.6	1183.6	*58%*	957.9	761.9	691.8	*72%*
	10	Water	1126.3	917.5	752.4	*67%*	511.3	390.1	203.7	*40%*	69.7	49.5	33.1	*48%*	231.2	182.5	164.6	*71%*	186.4	134.8	120.2	*64%*
	30		1365.9	1018.1	817.2	*60%*	705.3	423.3	266.1	*38%*	234.5	187.9	93.3	*40%*	269.9	225.8	193.7	*72%*	288.3	247.3	198.6	*69%*
	60		1251.9	829.0	630.2	*50%*	683.1	395.0	316.8	*46%*	361.5	192.1	60.3	*17%*	360.7	222.8	199.3	*55%*	349.1	283.0	222.0	*64%*
St. John's Lake	10	Sediment	2178.6	1962.3	1998.7	*92%*	871.6	543.6	466.4	*54%*	120.4	95.0	74.7	*62%*	704.4	462.0	442.4	*63%*	395.9	326.8	353.4	*89%*
	30		2079.2	1819.9	1566.4	*75%*	1396.8	956.8	724.4	*52%*	383.2	260.9	168.4	*44%*	1785.8	1285.6	1196.2	*67%*	651.1	537.4	482.7	*74%*
	60		2581.7	1841.3	1630.6	*63%*	1729.6	1202.0	965.0	*56%*	470.4	242.9	116.1	*25%*	1720.8	1148.3	1137.0	*66%*	776.6	517.3	468.4	*60%*
	10	Water	1041.2	891.1	697.6	*67%*	437.3	291.5	241.2	*55%*	106.8	82.5	60.7	*57%*	237.0	213.1	183.1	*77%*	156.2	141.8	125.2	*80%*
	30		2085.8	1935.3	1406.6	*67%*	869.4	602.8	345.0	*40%*	256.8	148.8	79.2	*31%*	487.0	340.6	298.4	*61%*	377.8	301.6	246.2	*65%*
	60		2241.3	1657.9	1370.7	*61%*	1275.7	893.9	601.2	*47%*	593.8	301.4	98.2	*17%*	740.5	508.7	444.4	*60%*	646.1	499.3	389.7	*60%*

Appendix 15 Butyltin body burdens in tissues of Hinia reticulata exposed to different sediments to show relative proportions in each tissue type and the proportion in each tissue derived from the sediment or the overlying water.

(All units are in ng. See section 6.3.2.2 for derivation of values).

Sediment type		Gill/Osphradium			Kidney			Digestive Gland			Head/Foot			Remaining tissue		
		Sediment	Water	Total	Sediment	Water	Total	Sediment	Water	Total	Sediment	Water	Total	Sediment	Water	Total
Talland Bay	**Total**	**101.5**	**64.1**	**165.6**	**91.9**	**34.8**	**126.7**	**223.1**	**152.1**	**375.2**	**446.0**	**234.1**	**680.1**	**330.4**	**196.0**	**526.4**
	% from each phase	*61.3%*	*38.7%*		*72.5%*	*27.5%*		*59.5%*	*40.5%*		*65.6%*	*34.4%*		*62.8%*	*37.2%*	
	% of body burden			*9%*			*7%*			*20%*			*36%*			*28%*
	TBT+DBT	**76.0**	**50.7**	**126.7**	**66.7**	**26.9**	**93.6**	**168.6**	**109.2**	**277.8**	**406.8**	**149.3**	**555.1**	**237.8**	**148.5**	**386.3**
	% from each phase	*60.0%*	*40.0%*		*71.2%*	*28.8%*		*60.7%*	*39.3%*		*73.1%*	*26.9%*		*61.6%*	*38.4%*	
	% of body burden			*9%*			*7%*			*19%*			*39%*			*27%*
	TBT	**57.1**	**34.9**	**92.0**	**38.0**	**17.4**	**55.4**	**75.7**	**66.0**	**141.7**	**334.7**	**121.8**	**456.5**	**214.7**	**116.1**	**330.9**
	% from each phase	*62.1%*	*37.9%*		*68.7%*	*31.3%*		*53.4%*	*46.6%*		*73.3%*	*26.7%*		*64.9%*	*35.1%*	
	% of body burden			*9%*			*5%*			*13%*			*42%*			*31%*
East Looe	**Total**	**16.7**	**8.3**	**25.1**	**5.6**	**3.0**	**8.5**	**12.0**	**13.5**	**25.5**	**84.7**	**16.3**	**101.1**	**22.9**	**13.7**	**36.6**
	% from each phase	*66.8%*	*33.2%*		*65.3%*	*34.7%*		*47.0%*	*53.0%*		*83.8%*	*16.2%*		*62.6%*	*37.4%*	
	% of body burden			*13%*			*4%*			*13%*			*51%*			*19%*
	TBT+DBT	**13.0**	**6.2**	**19.2**	**4.4**	**1.8**	**6.2**	**7.7**	**10.8**	**18.6**	**67.2**	**13.7**	**80.8**	**18.9**	**11.7**	**30.6**
	% from each phase	*67.7%*	*32.3%*		*71.2%*	*28.8%*		*41.6%*	*58.4%*		*83.1%*	*16.9%*		*61.6%*	*38.4%*	
	% of body burden			*12%*			*4%*			*12%*			*52%*			*20%*
	TBT	**11.8**	**5.0**	**16.8**	**3.5**	**1.1**	**4.6**	**5.8**	**5.4**	**11.2**	**60.3**	**11.7**	**72.0**	**18.1**	**9.4**	**27.6**
	% from each phase	*70.3%*	*29.7%*		*75.7%*	*24.3%*		*51.7%*	*48.3%*		*83.7%*	*16.3%*		*65.8%*	*34.2%*	
	% of body burden			*13%*			*3%*			*8%*			*54%*			*21%*
St.John's Lake	**Total**	**0.0**	**12.7**	**12.7**	**2.2**	**3.7**	**5.9**	**7.3**	**14.8**	**22.1**	**78.6**	**29.5**	**108.0**	**13.0**	**17.9**	**30.9**
	% from each phase	*0.0%*	*100.0%*		*37.8%*	*62.2%*		*33.0%*	*67.0%*		*72.7%*	*27.3%*		*42.0%*	*58.0%*	
	% of body burden			*7%*			*3%*			*12%*			*60%*			*17%*
	TBT+DBT	**0.0**	**11.8**	**11.8**	**1.5**	**2.5**	**4.0**	**6.5**	**8.6**	**15.1**	**57.2**	**20.6**	**77.8**	**11.2**	**14.3**	**25.5**
	% from each phase	*0.0%*	*100.0%*		*37.0%*	*63.0%*		*43.0%*	*57.0%*		*73.5%*	*26.5%*		*43.9%*	*56.1%*	
	% of body burden			*9%*			*3%*			*11%*			*58%*			*19%*
	TBT	**1.0**	**8.6**	**9.6**	**1.6**	**1.4**	**3.0**	**5.1**	**4.6**	**9.7**	**54.3**	**18.1**	**72.4**	**11.2**	**11.7**	**22.9**
	% from each phase	*10.2%*	*89.8%*		*52.4%*	*47.6%*		*53.0%*	*47.0%*		*75.1%*	*24.9%*		*49.0%*	*51.0%*	
	% of body burden			*8%*			*3%*			*8%*			*62%*			*19%*