APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 9, 327–334 (1995)

Short-term Bioconcentration and Distribution of Methylmercury, Tributyltin and Corresponding Inorganic Species in the Starfish *Leptasterias polaris*

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Starfish, *Leptasterias polaris*, were exposed between 30 min and 48 h to seawater containing 0.25 nmol dm^{-3} of radiolabelled methylmercury ($Me^{203}HgCl$), tributyltin [$(C_4H_9)_3{}^{113}SnCl$], and inorganic $^{203}HgCl_2$ and $^{113}SnCl_4$, with the objectives of comparing the uptake and distribution kinetics of these metal species in organs and tissues of treated organisms. Some starfish exposed to metals for 48 h were allowed to depurate for 24 h in clean seawater. Whole-body autoradiography was used to locate radiotracers very precisely within starfish tissues. The total amount of methylmercury (MeHg) accumulated in the whole animal after 48 h reached 0.53 nmol compared with 0.09 nmol for inorganic mercury, while tributyltin (TBT) reached 0.72 nmol compared with 0.017 nmol for inorganic tin. No significant reduction of body burdens occurred during the depuration period. The first-order rate constant characterizing the uptake of metals by whole animals, k_1, ranged from 0.102 h^{-1} for MeHg to 3.6×10^{-3} h^{-1} for inorganic mercury(II) and to 8.4×10^{-4} h^{-1} for inorganic tin(IV). The first-order rate constant characterizing the translocation of metals from seawater-exposed tissues toward internal organs, k_3, was available for inorganic Hg and Sn and had values similar to k_1. Concentration ratios between external tissues and internal organs after a 48 h exposure were 11.5 and 25.4 for MeHg and TBT, respectively, and 2.1 and 6.1 for inorganic mercury and tin. Furthermore, autoradiograms showed that MeHg and TBT were accumulated only on the external surface of the body wall and podia. This finding indicates a much slower translocation process for organometallic species than inorganic species, a process which seems to be related to the binding mode of MeHg and TBT to the organic matrix of external tissues of starfish.

Keywords: mercury; methylmercury; tin; tributyltin; bioconcentration; distribution; kinetics; starfish; *Leptasterias polaris*

INTRODUCTION

In a previous paper[1] on bioconcentration and distribution of inorganic mercury(II) and methylmercury (MeHg) in the starfish *Asterias rubens*, we observed that the uptake and distribution of MeHg and inorganic mercury differed after a 24 h exposure as three times more MeHg was accumulated, almost entirely on the external surface of the animals. Tributyltin (TBT) is another organometallic compound of great environmental concern which was, and is still, widely used in the marine environment as a biocide in antifouling paints.[2] High concentrations of this compound, particularly near highly frequented maritime routes and harbours, have been shown to be the cause of poor growth and shell malformations of oysters.[3,4] Despite many countries having regulated the use of tin antifouling paints,[5,6] recent measurements showed that TBT concentrations are still high in some aquatic environments and the occurrence of sublethal effects on the aquatic fauna is often reported.[7–10]

Although both TBT and MeHg belong to the broad family of alkylmetal compounds, their physical and chemical properties differ greatly. TBT bears three n-butyl groups instead of a single methyl group and thus has a molar volume (294 cm^3 mol^{-1}) (as calculated by the method of

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CCC 0268–2605/95/040327–08

Received 4 August 1994
Accepted 20 January 1995

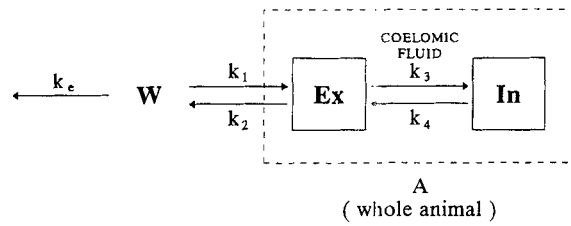
LeBas[11]) seven times higher than MeHg (41 $cm^3 mol^{-1}$). TBT complexes are tetra- and penta-coordinated[12] while unidentate MeHg complexes are usually linear.[13] TBT solubility in seawater is very low (octanol–water partition coefficient, $K_{ow} = 5000–6300$)[14] while MeHg is quite soluble ($K_{ow} = 1.7 \pm 0.2$)[15] due to the formation of chloro complexes. These differences should be reflected by differences in their bioconcentration and distribution in biota. However, no previous study has simultaneously compared their uptake and distribution in the same organisms kept under controlled environmental conditions.

The purpose of this work was to compare short-term (48 h) bioconcentratations and distributions of MeHg, TBT, and inorganic mercury(II) and tin(IV) in *Leptasterias polaris*, a six-armed starfish of the Asteridae family. Starfish was chosen as a biological model because of its wide distribution in estuarine and shallow coastal regions often threatened by contaminated effluents and its quick acclimatization to laboratory conditions.[16] It has also been shown that starfish may play an important role in the trace-metal balance of benthic ecosystems.[17]

MATERIALS AND METHODS

Radiolabelling experiments

Metal species, radioactive markers (^{203}Hg and ^{113}Sn) and experimental conditions have been previously described.[18] Starfish were caught by scuba diving in the St Lawrence Estuary, Canada, and were acclimatized to laboratory conditions for three days before experiments. The mean body weight of the starfish was 79 ± 28 g. Exposure to metals was performed in 4 dm^3 beakers containing 3.5 dm^3 of UV-sterilized aerated natural seawater (temperature 5 ± 1 °C, salinity 25.9 ± 08‰) spiked with 0.25 nmol dm^{-3} of metal solutions 15 min before the immersion of animals. Exposure periods were 0.5, 1.5, 4, 8, 16, 24 or 48 h. Some starfish exposed for 48 h were allowed to depurate in uncontaminated seawater for 8, 16 and 24 h. Three starfish were collected at each sampling time and briefly rinsed in clean seawater upon sampling. Organs and tissues were then dissected, weighed and their radioactivity determined by gamma counting with an LKB 1272 Clinigamma® counter. Radioactivity counts were corrected for background level and the decay of isotopes, and converted to nmol of Hg or Sn. At $t = 8$ and 48 h and after 24 h of depuration, two additional starfish were used for whole-body autoradiography.[18,19] Due to the limited amount of $(C_4H_9)_3{}^{113}SnCl$ available, only four starfish were exposed for 48 h to tributyltin, two of them being used for quantitative measurements and the other two being devoted to whole-body autoradiography.



Scheme 1 Compartmental model for the bioconcentration of metal species by the starfish.

Kinetics

A first-order kinetic model was used to describe exchanges of MeHg and inorganic metal ions between seawater, **W**, and the whole animal, **A**, and between tissues directly exposed to seawater (body wall, podia and mouth), **Ex**, and internal organs (pyloric caeca, gonads, and stomach), **In** (Scheme 1). Coelomic fluid (seawater filling the general cavity of the starfish) is not included in the compartment **In** as that fluid was considered to act only as a means of transportation for dissolved metals from compartment **Ex** to **In** and was not a site for accumulation. The first-order rate constants k_1, k_2, k_3 and k_4 characterize exchanges between compartments and k_e is the first-order rate constant characterizing the loss of bioavailable metals from water by various processes (adsorption, volatilization, complexation and chemical degradation).

A preliminary analysis of our experimental results revealed no significant loss of metals from whole animals and very low exchanges between compartments during the 24 h depuration period. We then considered k_2 and k_4 (Scheme 1) to be very small and negligible for this experiment. Thus, the model was simplified to that shown in Scheme 2.

$$\overset{k_e}{\leftarrow} \mathbf{W} \overset{k_1}{\rightarrow} \underset{\mathbf{A}}{\underbrace{\mathbf{Ex} \overset{k_3}{\rightarrow} \mathbf{In}}}$$

Scheme 2

Rate expressions for the transfer of a given organometallic or inorganic species from water to the whole animal can be written as in Eqns [1] and [2]:

$$\frac{d\mathbf{W}}{dt} = -k_1\mathbf{W} - k_e\mathbf{W} = -(k_1 + k_e)\mathbf{W}, \quad [1]$$

$$\frac{d\mathbf{A}}{dt} = k_1\mathbf{W}, \quad [2]$$

where **W** and **A** are quantities of metals in seawater and in the whole animal, respectively. Integrated forms of these rate equations are given by Eqns [3] and [4].

$$\mathbf{W} = \mathbf{W}_0 \exp[-(k_1 + k_e)t] \quad [3]$$

$$\mathbf{A} = \frac{k_1\mathbf{W}_0}{k_1 + k_e}\{1 - \exp[-(k_1 + k_e)t]\} \quad [4]$$

where $\mathbf{W}_0$ is the initial amount of a given metal in seawater (0.875 nmol), assumed to be 100% bioavailable at $t = 0$. Equation [4] was used to calculate experimental values of k_1 and k_e by nonlinear regression analysis (STATGRAPH®) rather than Eqn [3] because **W** represented the quantity of bioavailable metal in seawater and was not directly available from our experimental data. Measurements of radioactivity at time t would give the total amount of the metal in seawater, which might include some non-bioavailable chemical forms of the metal.

As the whole animal was formed by two distinct compartments, **Ex** and **In**, their exchange rate equations can be expressed as in Eqns [5] and [6]:

$$\frac{d\mathbf{Ex}}{dt} = k_1\mathbf{W} - k_3\mathbf{Ex} \quad [5]$$

$$\frac{d\mathbf{In}}{dt} = k_3\mathbf{Ex} \quad [6]$$

where **Ex** and **In** are the quantities of metal in external tissues and internal organs, respectively. Integrated forms of these rate equations are as in Eqns [7] and [8].

$$\mathbf{Ex} = \frac{k_1\mathbf{W}_0}{(k_3 - k_1 - k_e)}\{\exp[-(k_1 + k_e)t] - \exp[-k_3t]\} \quad [7]$$

$$\mathbf{In} = \mathbf{W}_0\left\{\frac{k_1}{(k_1 + k_e)}[1 - \exp-(k_1 + k_e)t] - \frac{k_1}{(k_3 - k_1 - k_e)}[\exp-(k_1 + k_e)t - \exp-k_3t]\right\} \quad [8]$$

We used Eqn [8] to evaluate k_3 by nonlinear regression analysis, using values of k_1 and k_e from Eqn [4].

RESULTS

Data obtained for the bioconcentration and the depuration of MeHg and inorganic metal species are presented in Fig. 1. Data are shown for the whole animal (**A**) and internal organs (**In**). As internal organs accounted for only a small proportion (<10%) of total body burdens, data for external tissues (**Ex**) had values very comparable with those of **A** and are not illustrated.

Starfish accumulated nearly 0.09 nmol of inorganic mercury(II) after 48 h; internal organs and coelomic fluid represented 9% and 2%, respectively, of the mercury body burden, while external tissues accounted for the remaining 89%. The value of the rate constant k_1 was similar to the value of k_3 (Table 1). However, an unexpected uptake pattern was noted at the beginning of the exposure as contents in the whole animal and internal organs increased very rapidly during the first 30 min and decreased in the following 4 h, while continuously increasing thereafter. This behavior resulted in relatively low r^2 values for fitted curves of inorganic mercury(II) (Table 1). The variability of Hg content during the depuration phase was rather high and does not allow a clear trend to be distinguished.

Bioconcentration curves of inorganic tin(IV) exhibited high values of r^2 (Table 1) and quantities observed in the whole animal increased at a slower rate, reaching 0.017 nmol tin after 48 h, a value five times lower than that for inorganic mercury (Fig. 1). Inorganic tin was detected in

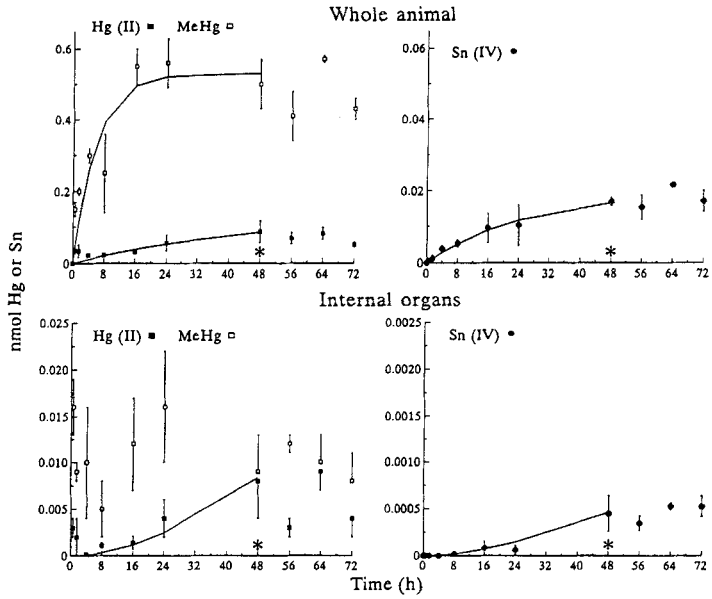


Figure 1 Quantity of metals (nmol) bioconcentrated by *L. polaris* exposed for 48 h to 0.25 nmol dm^{-3} of MeHg, inorganic mercury(II), and inorganic tin(IV). The star indicates the beginning of the depuration period. Points represent means ± S.D., $n = 3$. Fitted curves were obtained by nonlinear regression analysis using Eqn [4] for the whole animal, and Eqn [8] for the internal organs.

internal organs 16 h after the beginning of exposure and reached only 0.45 pmol after 48 h, which represented less than 3% of the total body burden. Tissues in direct contact with contaminated seawater and coelomic liquid represented 90% and 7% of the total tin body burden, respectively. Values of rate constants k_1 and k_3 were almost equal and were about four times lower than the corresponding values for inorganic mercury (Table 1). No significant change was observed during the depuration period.

Bioconcentration of MeHg in whole starfish reached a plateau after 16 h of exposure, at 0.53 nmol, which represented approximately six times the quantity of mercury accumulated by starfish exposed to inorganic mercury for 48 h (Fig. 1). The rate constant k_1 was 34 times higher than the value found for inorganic mercury (Table 1). The value of r^2 for the whole animal was high. However, the MeHg content of internal organs was characterized by a very high variability. It was not possible to define any quantitative trend and k_3 was not evaluated. The quantity of MeHg of internal organs reached approximately 0.01 nmol, a value representing only 2% of the whole body burden and quite similar to the maximum value reached by inorganic mercury with a similar to the maximum value reached by inorganic mercury with a similar exposure time. The MeHg content of the whole animal and internal organs did not change during the depuration period.

Starfish (two) used to quantify the uptake of TBT accumulated 0.72 ± 0.07 nmol after a 48 h

Table 1 Values of r^2 for fitted curves of Fig. 1 and rate constants k_e, k_1 and k_3 calculated for the 48 h bioconcentration of MeHg and inorganic mercury (II) and tin(IV) in the starfish *L. polaris*

	r^2		Rate constant ± S.E.M. (h^{-1})		
	Whole animal (**A**)	Internal organs (**In**)	k_e	k_1	k_3
Mercury(II)	0.55	0.55	0.024 ± 0.030	0.0036 ± 0.0017	0.0037 ± 0.0002
MeHg	0.84	n.d.[a]	0.067 ± 0.035	0.102 ± 0.034	n.d.[a]
Tin(IV)	0.99	0.96	0.036 ± 0.007	0.00084 ± 0.00008	0.00084 ± 0.00002

[a] n.d., not determined.

Table 2 Concentrations of MeHg, TBT and inorganic mercury(II) and tin(IV) in external tissues, [Ex], and internal organs, [In], of the starfish *L. polaris*, and their ratio after a 48 h exposure[a]

	[Ex][b]	[In][b]	[Ex]/[In]
Mercury(II)	1.53 ± 0.42	0.88 ± 0.42	2.1 ± 0.8
MeHg	10.34 ± 4.39	0.96 ± 0.46	11.5 ± 3.0
Tin(IV)	0.30 ± 0.06	0.05 ± 0.01	6.1 ± 1.9
TBT	13.73 ± 0.55	0.57 ± 0.12	25.4 ± 6.1

[a] Data are means ± S.D., $n = 3$.
[b] [ ], Concentrations in pmol Hg g^{-1} or pmol Sn g^{-1} (wet weight).

exposure, a quantity higher than for MeHg. The amount of TBT accumulated in internal organs reached 0.009 ± 0.002 nmol, a level similar to those of both mercury species, and represented 1.2% of the whole-body burden. External tissues represented nearly 99% of the whole-body burden of TBT and only 0.1% was found in the coelomic fluid.

Concentrations in external tissues, [Ex], in internal organs, [In], and [Ex]/[In] ratios for starfish exposed to metal species for 48 h are given in Table 2. Relative concentrations in external tissues, [Ex], reflected the relative body burdens. However, the concentration of metals in internal organs, [In], was similar for both mercury species and TBT, resulting in concentration gradients between external tissues and internal organs, as illustrated by the ratio [Ex]/[In], decreasing in the order TBT > MeHg > Sn(IV) > Hg(II).

We also examined the distribution of mercury and tin species in some particular tissues after 48 h of exposure. Data shown in Fig. 2 represent the distribution of metal species in tissues and organs forming compartments **Ex** and **In**. The stomach accounted for 12–15% of the inorganic mercury, MeHg and TBT internal contents while the proportions of these species varied from 34 to 47% for pyloric caeca and from 31 to 41% for gonads. Coelomic fluid accounted for approximately 20 and 7% of the inorganic mercury and TBT internal contents, respectively. However, coelomic fluid contained more than 71% of the internal content of inorganic tin, leaving only minor contributions for stomach (4%), pyloric caeca (21%) and gonads (4%) when compared with the other metal species. The mouth is a small organ which accounted for only 1–5% of the metal content of external tissues. Relative contents of TBT and MeHg in body wall were higher than in podia, while inorganic mercury and tin seem to exhibit a different pattern.

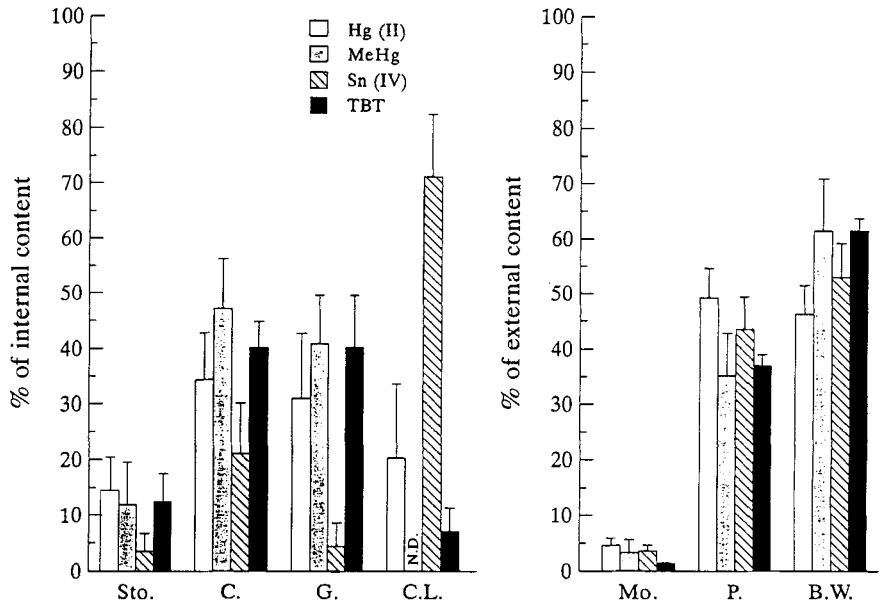


Figure 2 Distribution of MeHg, TBT, inorganic mercury(II) and inorganic tin(IV) in the tissues of *L. polaris* after a 48 h exposure to 0.25 nmol dm^{-3}. Values for stomach (Sto.), pyloric caeca (C.), gonads (G.) and coelomic liquid (C.L.) are percentages of the metal found in the internal compartment (internal organs + coelomic liquid) and values for mouth (Mo.), podia (P.) and body wall (B.W.) are percentages of the metal in the external compartment. Values are means ± S.D., $n = 3$. N.D., not determined.

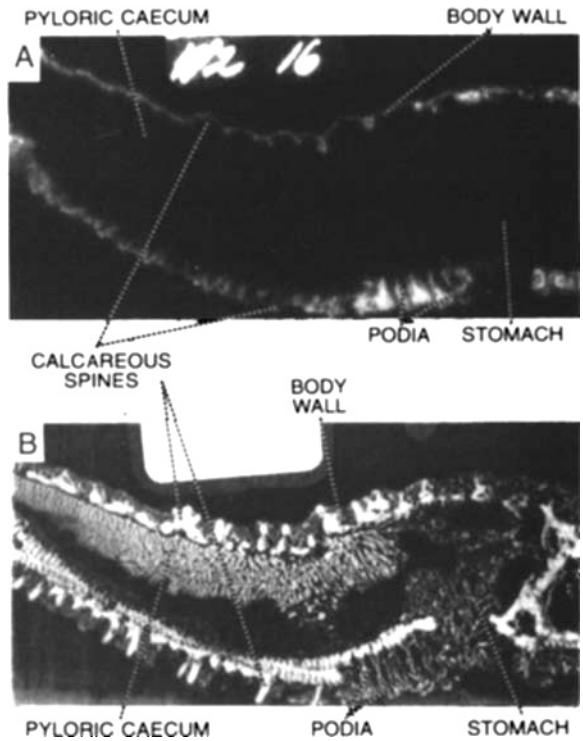


Figure 3 (A) Whole-body autoradiogram of a transverse section of *L. polaris* exposed for 48 h to 0.25 nmol dm^{-3} of TBT. (B) Microphotograph of the corresponding tissue section.

The autoradiogram taken from the starfish exposed to TBT (Fig. 3) shows that labelling of the body wall is restricted to the external epidermis. Outer surfaces of podia and of calcareous spines also show higher radioactivity compared with their inner parts. Similarly the autoradiogram from starfish exposed to MeHg (Fig. 4) shows labelling limited to the external surface of the body wall, as observed for TBT.

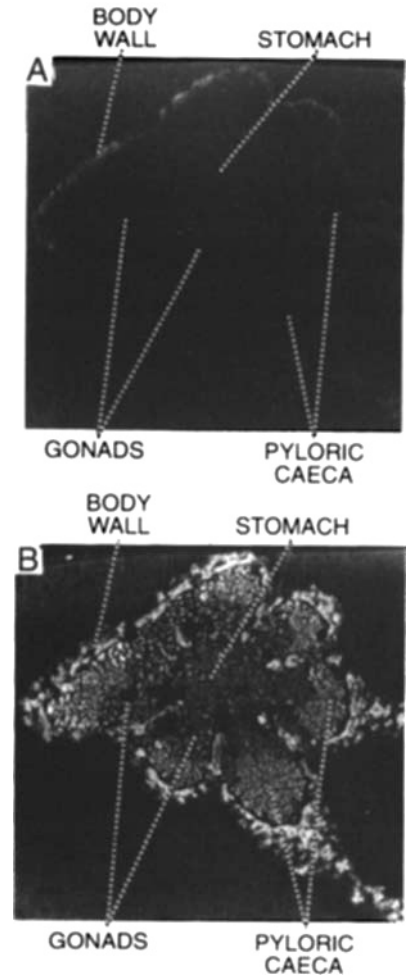


Figure 4 (A) Whole-body autoradiogram of a radial section of *L. polaris* exposed for 48 h to 0.25 nmol dm^{-3} of MeHg. (B) Microphotograph of the corresponding tissue section.

DISCUSSION

The validity of the bioconcentration model is first based on the assumption that coelomic fluid acts mainly as a means of transportation and not as an accumulation site. Our results confirmed that coelomic fluid is not an uptake site as the percentage of inorganic mercury and TBT body burdens in the coelomic fluid were very low (1.7% and 0.1% respectively). The very low accumulation of MeHg, as well as inorganic mercury, in the coelomic fluid was illustrated in a previous experiment[1] with another starfish species, *Asteria rubens*. Thus, the MeHg content of the coelomic fluid of *L. polaris* for this experiment was probably low, although not determined.

In the present state of its development, our kinetic model cannot take into account speciation changes of metal ions during the course of the exposure. Changes in the speciation of inorganic mercury, MeHg and TBT are not likely to have taken place during this short-term experiment,[18,20] while inorganic tin must be considered as an unusual case, to be treated with caution. Tin tetrachloride ($SnCl_4$) is present at high chloride concentrations ($[Cl^-] \geq 0.9$ M) as a hexachlorostannate anion. At a lower chloride concentration, this anion is rapidly hydrolysed to various forms of hydrated tin(IV) oxide, a very

insoluble and unreactive species.[21] As the salinity of the seawater used was 26‰ ($[Cl^-] \approx 0.45$ M) and the fluid filling the starfish internal cavity is iso-osmotic with seawater, such a change in speciation is likely to have occurred during the course of our experiment. The higher proportion of tin contained in the coelomic fluid and the lower tin content of internal organs (Fig. 2) are indicative that inorganic tin in the coelomic fluid was not transferred to internal organs to the same extent as was observed with other metal species. Conceivably, a change in its speciation may have rendered inorganic tin(IV) less available to uptake by internal organs from the coelomic fluid. The very low whole-body accumulation and the low uptake rate of inorganic tin in *L. polaris* are also probably due to its low bioavailability, resulting from the chemical inertia of SnO_2.

Our simplified kinetic model adequately described the bioconcentration of MeHg and inorganic tin(IV) and has the advantage of accounting for the loss of bioavailable metal ions. In such a model, the uptake plateau observed for MeHg (Fig. 1) resulted from the exhaustion of seawater in bioavailable metal species. This model should be used with care for longer exposure periods as rates of reverse reactions (k_2 and k_4) are likely to become important. This model does not account for the unexpected and apparently erratic uptake pattern of inorganic mercury. To our knowledge, this has not been reported before for starfish or other aquatic organisms. Mechanisms for such an uptake process are not clear at the moment but might be related to some modifications of membrane properties induced by inorganic mercury[22] or to some competitive phenomena for adsorption sites between mercury and other ions in seawater. Further work is needed to study in detail the bioconcentration kinetics of inorganic mercury on a very short timescale.

The uptake and distribution of inorganic mercury and MeHg in *L. polaris* at the end of the exposure showed features similar to those observed previously for *A. rubens*,[1] i.e. a higher accumulation of MeHg versus inorganic mercury and a distribution restricted to external surfaces. The higher uptake rate and the higher burden reached by MeHg are related to its exceptional affinity for sulfhydryl groups of living tissues. The higher value of k_e for MeHg (Table 1) is probably related to its relatively high volatility.[13] The high accumulation of TBT is probably related to its lipophilicity and its capacity to bind ligands present on living tissues.[23]

Autoradiograms showed that labelling of tissues by radioactive MeHg and TBT was mostly restricted to seawater-exposed external surfaces. This direct observation of metal deposition, when coupled to the higher concentration ratios between outer and inner tissues, confirms that translocation of both MeHg and TBT is a more difficult process compared with that of inorganic mercury and tin. Starfish are poikilosmotic organisms (i.e. coelomic fluid is not osmoregulated[24]) and metallic ions, such as inorganic Hg, are likely to be translocated more easily than other chemical species resulting in low value of **[Ex]/[In]**. High steric hindrance and low solubility in water decreases the accumulation of chemicals by aquatic organisms.[25] These chemical properties can be invoked to explain the slow translocation of TBT in *L. polaris*, but this explanation does not hold for MeHg, a small linear water-soluble molecule expected to be translocated easily in the starfish. Surprisingly, MeHg and TBT added to food were translocated rather easily across the walls of pyloric caeca toward tissues constituting body walls and podia.[18] Such a difference in the translocation processes of MeHg and TBT in the starfish, as a function of the route of uptake might indicate that MeHg and TBT need to be associated with organic molecules, such as nutrients and other organic molecules abundant in the coelomic fluid of echinoderms during digestion,[26–28] to be transported through organs or tissues.

CONCLUSION

The main observation of this study is the trapping of dissolved organometals (TBT and MeHg) on the external tissues and their very slow translocation inside studied organisms. Such a mechanism (probably related to the presence of organic mucus on the body wall and podia of starfish) might represent an efficient protection against the toxic effects of dissolved TBT and MeHg, presumably present in contaminated sediments and overlying seawater. When compared with the rapid rate at which these organometals are translocated toward sensitive organs (such as gonads) when ingested with food,[20] it becomes clear that the trophic route of contamination of benthic invertebrates by organometals is much more important than the direct uptake from contaminated seawater.

Even if bioconcentration and distribution of both organometallic ions were qualitatively similar, the higher value of the **[Ex]/[In]** ratio of TBT indicates that its rate of translocation was slow compared with that of MeHg. Differences in the translocation rate between these two organometals might be attributed to differences in their physical properties: translocation through body wall and podia up to the coelomic fluid is likely to be more difficult for bigger and less water-soluble TBT molecules.

MeHg and TBT represent only two members of the organometallic family. Much more work is needed to establish quantitative relationships between the environmental fate of organometals and their structural, physical and chemical properties. New organometals are being developed by the chemical and pharmaceutical industries,[29–31] and to be able to predict the fate of these new compounds in aquatic ecosystems will become important.

Acknowledgements This work was supported by the National Science and Engineering Research Council of Canada, the Fond FCAR (Québec) and the Swedish Environment Protection Board. The authors are grateful to Agneta Boström, Claire Labrie and Johanne Noël for their technical assistance. This publication is a contribution of the Oceanographic Centre of Rimouski, a partnership of INRS (Institut National de la Recherche Scientifique) and UQAR (Université du Québec à Rimouski) operating under the auspices of the University of Québec.

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