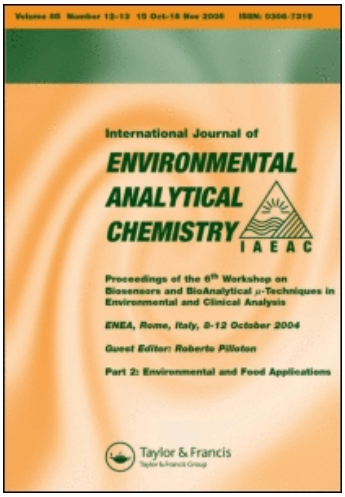

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A simple model to predict compound loss processes in aquatic ecotoxicological tests: calculated and measured triphenyltin levels in water and biota

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A simple model to predict compound loss processes in aquatic ecotoxicological tests: calculated and measured triphenyltin levels in water and biota

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A new simple model, based on the fugacity approach, has been developed to provide a predictive tool useful in the planning phase of aquatic ecotoxicological tests for assessing the actual daily concentrations in water. In our experiments, three nominal concentrations (100, 225, and 500 ng L^{-1}) of triphenyltin chloride (TPT-Cl) were employed for an exposure period of 28 days in 50 L aquaria with the echinoderm *Antedon mediterranea* as test species. Extracts from water and biota samples collected during the experiments were analysed by GC-MS/MS, after the extraction/derivatization step. An indicative mean BCF (V/V) on a fresh weight base of $3.5 \times 10^4 \pm 0.8 \times 10^4$ (standard deviation) could be calculated for *A. mediterranea*. Three different compartments (air, water and biota) and main advection/reaction processes are taken into account in the model design, and the comparison between predicted and measured concentrations in both water and biota for the three concentrations tested confirmed that the assumptions given in our model application were valid and useful for further applications.

Keywords: TPT-Cl analysis; Ecotoxicological tests; Modelling; Loss processes

1. Introduction

The analytical determination of the water exposure level is a key point and a primary prerequisite in ecotoxicological aquatic tests, as recommended by the Technical Guideline Documents of the European Commission [1]. During medium- and long-term tests, it is not easy to maintain the actual medium concentrations constant and close to the nominal ones, especially with highly hydrophobic or reactive molecules [2]. In view of this, it would be of great help to employ a model which can predict actual concentrations based on both the physico-chemical properties of the compounds and the

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environmental parameters of the system. The experimental analytical data often show that the exposure concentrations, far from being constant, tend to increase or decrease depending on the number of ongoing processes in the system and on the experimental conditions (static, semi-static, or flow-through), even if recommended standard precautions are adopted [2]. In a standard aquarium exposure system, a number of different loss processes may occur. These involve both abiotic processes (such as volatilization, unspecific adsorption to the glass, plastic, and rubber materials, and chemical degradation) and biotic processes (such as bioconcentration and biotransformation). All these events are controlled by several factors: (a) the physico-chemical properties of the compound, (b) the system parameters, and (c) the physiological characteristics of the organisms. It is known that the physico-chemical properties are very important for environmental partitioning and fate. They indicate the relevance of several processes such as volatilization and bioconcentration [3]. Environmental characteristics such as compartment volumes, composition, temperature, salinity, and input and output flows are essential factors for environmental distribution [4]. The organism as well is very important in terms of volume, exchange total surface, metabolism, and composition (e.g. lipid fraction). The possibility of predicting the extent of the different loss processes and forecasting the actual exposure concentrations before running the experiment can be a great advantage in the planning phase of an ecotoxicological test. This can lead to an accurate choice of the appropriate experimental conditions such as the water/animal volume ratio or the compound renewal rate. Predictive models are currently used in risk-assessment procedures for predicting environmental concentrations in a variety of ecosystems [1] and can be adapted to the microecosystem level [5]. In the present article, a simple model derived from the Mackay Fugacity Model [4] has been developed for aquatic exposure experiments and validated with analytical data on triphenyltin compounds (TPT) and *Antedon mediterranea*. This echinoderm is a common Mediterranean crinoid, which represents an already-established test species that has been successfully used in ecotoxicological tests for endocrine disrupters [6, 7].

Triphenyltin compounds have been used as broad-spectrum agricultural fungicides (mainly as triphenyltin-hydroxide and triphenyltin acetate) since the early 1960s to combat a range of fungal diseases in various crops [8, 9]. In addition, triphenyltin compounds were extensively used in antifouling paints and caused water pollution in aquatic ecosystems [10]. From the results of such multiple employments, triphenyltin compounds may be widely detected in abiotic and biotic compartments, both in freshwater and in marine ecosystems, from non-detectable levels up to hundreds of $ng\,L^{-1}$ and thousands of $ng\,g^{-1}$ d.w. in water and sediment-biota, respectively [11–18]. At present, the use of most TPT compounds is severely restricted because of their previously documented environmental persistence [19] and the available toxicological data on their possible endocrine-disrupting activity (androgenic effects) on the reproductive biology of many animals [20–27]. TPT-Cl is one of the compounds being tested in the ongoing COMPRENDO EU project [28].

In the present article, a very simple model is proposed, derived from the Level II of the fugacity model [4], and takes into account three compartments (air, water, and biota) and the major loss mechanisms, such as advection and reaction. Compound addition is considered as well, coming from the compound renewal throughout the water changes or from the air–water exchange. Predicted concentrations are calculated and compared with those actually measured in water and biota compartments, for the overall exposure period (28 days).

2. Experimental

2.1 *Exposure experimental set-up*

The exposure experiments were performed in 50 L glass aquaria of artificial sea water on the echinoderm *Antedon mediterranea*. Three different nominal concentrations, of TPT-Cl, 100, 225, and 500 ng L^{-1}, were tested in separate exposure aquaria in parallel with control experiments performed in control and solvent-control aquaria. The experimental specimens were collected from the Tirrenian Italian coast (Giglio Island). The exposure period lasted 28 days. The aquaria were maintained in semi-static conditions, with a daily partial water renewal of 20% of water volume (meaning a total water renewal in 5 days). During the exposure period, both water and biota samples were collected at the different prefixed times: from time 0 (immediately after the beginning of the exposure experiment) up to 28 days. Water samples were acidified to pH 4 with 10% HNO_3 and stored in HDPE jars at 4°C until analyses were performed. After fresh-weight evaluation, tissue samples were freeze-dried for 24 h (Freeze dryer Pirani 1001, Edwards, Boston, MA), ground, weighed again, and stored in HDPE jars at −20°C until analysis. TPT was analysed in 39 water and 10 biota samples from the exposure tanks. Since the main target of the research work is to determine the possible effects of TPT at the biological level, the number of biota samples processed for chemical analyses was limited to save most of the collected samples for the parallel biological analyses [29]. Physico-chemical water parameters, such as temperature, density, pH, nitrite and nitrate levels, and hardness were controlled using standard methods and common laboratory equipment. All the measured parameters were within acceptable ranges (except for nitrite and nitrate levels, sometimes above the limit). Mortality was limited (far below 20%) and casually distributed (G-test statistic).

2.2 *Analytical procedures*

2.2.1. Chemicals. Triphenyltin (TPT) chloride was purchased from STREM Chemicals (Bischheim, France). 2,2,4-Trimethylpentane (VWR, France) was used in GC/MS/MS. Sodium tetraethylborate, min. 98% (STREM Chemicals, Bischheim, France), 99+% glacial acetic acid (Lancaster, Bischheim, France), and ammonium acetate 98% (Lancaster, Bischheim, France) were used for the derivatization procedure. Methanol (JT Baker, France) was used for the extraction of *A. mediterranea* tissues.

2.2.2. Equipment. GC/MS/MS analyses were performed using a Thermoquest (Les Ulis, France) system consisting of a Trace GC 2000 gas chromatograph equipped with a large-volume injection-programmed temperature vaporizer (LVI-PTV) split–splitless injector, an AS 2000 autosampler, and a POLARIS Q ion-trap mass spectrometer (Thermofinnigan, Les Ulis, France). For data processing, Excalibur software from Thermofinnigan was used. The injector was equipped with a 12 cm × 2 mm i.d. Silcoseeve liner (Thermofinnigan). For water samples, 60 µL of extract was injected onto the LVI-PTV injector in constant flow mode at 1 mL min^{-1} and at an injection rate of 10 µL s^{-1}. The temperature of the injector was initially set at 70°C then increased, first to 90°C at 5°C s^{-1} (evaporation phase, 0.2 min) and then to 280°C at 10°C s^{-1} (transfer phase), where it was maintained for 1 min. The injector

temperature was increased to 320°C and maintained for 5 min for the cleaning phase, where the split flow was set at 200 mL min^{-1}. The PTV split/splitless valve was operating in splitless mode during the entire transfer phase, and the solvent valve temperature was set at 100°C. For *A. mediterranea* samples, 2 μL of extract was injected onto the PTV injector in constant flow mode at 1 mL min^{-1}. The temperature of the injector was initially set at 85°C, then increased to 300°C at 10°C s^{-1} (transfer phase), where it was maintained for 1.5 min. The injector temperature was increased to 320°C and maintained for 5 min for the cleaning phase, where the split flow was set at 50 mL min^{-1}. The PTV split/splitless valve was operating in splitless mode during the entire transfer phase. Phenyltin compounds were separated on a 30 m × 0.25 mm i.d. column coated with 0.25 μm of 65% dimethyl–35% phenyl polysiloxane phase (BPX-35, SGE, Courtaboeuf, France). The temperature of the column was initially set at 100°C for a period of 2.5 min, then increased at different rates and with four ramps to 270°C. Helium was used as the carrier gas at a constant flow of 1 mL min^{-1}. The transfer line was set at 300°C with the external ion source at 280°C. The ions in the electronic impact (EI) for the target TPT species (m/z 351 + 197) were selected and fragmented with helium gas (collision-induced dissociation) in the ion trap. The second-order mass spectra resulting from the most intense fragment were scanned from m/z ion 50 to the mass of the selected ions (transition m/z 351 − 197). The concentrations were calculated using the calibration curves established for each compound in internal standardization mode with tripropyltin and diheptyltin as internal standards.

2.2.3. Extraction/Derivatization. Two grams of *A. mediterranea* tissues were submitted to microwave extraction at 70 W for 3 min in a closed vessel, with 30 mL of an acetic acid/methanol (3/1) mixture. The derivatization procedure consisted of mixing 5 mL of biota extract or 100 mL of water (from the aquaria) with 1 mL of NaBEt4 (2%) at pH 4.8 (100 mL of acetate buffer 1.2 M). In the case of water samples, the triphenyltin species was recovered in 5 mL of 2,2,4-trimethylpentane, evaporated to 1 mL under gentle nitrogen flow, before analysis by LVI-PTV-GC-MS/MS. For *A. mediterranea* tissues, it was recovered in 1 mL of 2,2,4-trimethylpentane and directly analysed by GC-MS/MS.

2.2.4. TPT recoveries and method performance. The TPT limit of quantification was 2 ng L^{-1} in water samples and 24 ng g^{-1} in *A. mediterranea* tissues. For the latter, extraction recovery was 120% (RSD 15% with three replicates) after spiking 500 mg of biota tissues with 200 ng g^{-1} of TPT.

2.3 *The model*

The model, derived from the Level II Mackay fugacity model [4], requires a few input parameters: the physico-chemical properties of the compounds and the main environmental characteristics. Volatilization, chemical, and photochemical reactions together with bioconcentration and biotransformation are the main processes affecting the compound concentration and fate in the experimental aquaria. The physico-chemical properties of TPT-Cl are reported in table 1, and the main characteristics of the experimental system in table 2. The time trends of the concentrations in water and in

Table 1. Physico-chemical properties of TPT-Cl.

Property	Value	Unit
Molecular weight [30]	385.5	$g\,mol^{-1}$
Water solubility [30]	1.0	$mg\,L^{-1}$
Vapour pressure at 20°C [19]	3.8×10^{-9}	Pa
Henry's constant[a]	1.46×10^{-6}	$Pa\,m^3\,mol^{-1}$
Kow [30]	2690	
BCF on fresh weight[b]	3.6×10^4	
$t_{1/2}$ in air [19]	93	days
$t_{1/2}$ in water [19]	111	days
$t_{1/2}$ in biota[c]	30	days

[a]Calculated as follows: vapour pressure (Pa)/water solubility ($mol\,m^{-3}$). [b]Experimental datum on *Mytilus edulis* [34]. [c]Datum estimated by the authors.

Table 2. Main characteristics of the experimental system.

Characteristic	Value	Unit
Water volume in one aquarium	50	L
Volume of each water change	5	L
Air volume in one aquarium	10	L
Air volume in the room	63,000	L
Number of animals in each aquarium[a]	44–52	
Fresh weight of one animal[b]	0.85	g
Animal density[c]	1.2	$g\,mL^{-1}$

[a]Each aquarium had a different initial number of animals as follows: 51, 52, and 44 animals in the 100, 225, and $500\,ng\,L^{-1}$ nominal exposure aquaria, respectively. [b]Mean datum on 15 cases (0.18 g standard deviation and 0.62–1.08 g minimum–maximum interval). [c]Datum estimated by the authors.

the biota (unsteady-state condition) were obtained by applying the mass balance equation, reported below, in a suitable series for the exposure period (28 days):

$$M_{\mathrm{tot}(t)} = f_t[(Z_a V_a) + (Z_w V_w) + (Z_b V_b)],$$

where $M_{\mathrm{tot}(t)}$ = total moles in the system at time t (mol); f_t = fugacity in the system at time t (Pa); Z_a = capacity of the air compartment = $1/(RT)$ (R = gas constant = $8.314\,m^3\,Pa\,mol^{-1}\,K^{-1}$; T = absolute temperature in K) ($mol\,m^{-3}\,Pa^{-1}$); Z_w = capacity of the water compartment = 1/H (H = Henry's constant) ($mol\,m^{-3}\,Pa^{-1}$); Z_b = capacity of the biota compartment = BCF/H (BCF = bioconcentration factor) ($mol\,m^{-3}\,Pa^{-1}$); V_a = volume of the air compartment (m^3); V_w = volume of the water compartment (m^3); and V_b = volume of the biota compartment (m^3).

$M_{\mathrm{tot}(t)}$ values initially introduced in water at time 0, are deduced by the nominal concentrations and the water volume. Then, $M_{\mathrm{tot}(t)}$ values are calculated by adding to the remaining moles those of new inputs (advection in the system by air and water) and by subtracting those eliminated throughout loss processes (advection out the system by air, water, and biota, and reaction in the three compartments). Advection by air is possible, both inside and outside the system, especially during the water renewal because of the daily opening of the aquarium and the consequent mixing of the air of the aquarium with that of the room. Advection by water, both inside and outside the system,

can be calculated more easily since it consists of the moles of compound subtracted or added during the water renewal. Advection by biota only occurs outside the system, when the exposed animals inside the aquarium are collected or removed. All these processes are described algebraically by the following input–output mass balance equation:

$$\begin{aligned} M_{\mathrm{tot}(t)} = {} & (M_{\mathrm{a}(t-1)}\mathrm{e}^{-K_{\mathrm{a}}\Delta t}) + (M_{\mathrm{w}(t-1)}\mathrm{e}^{-K_{\mathrm{w}}\Delta t}) + (M_{\mathrm{b}(t-1)}\mathrm{e}^{-K_{\mathrm{b}}\Delta t}) \\ & - M_{\mathrm{a}(t-1)}n_{\mathrm{w}(t-1)} + \left[\left(\frac{M_{\mathrm{a-500\ ng/L}(t-1)}}{V_{\mathrm{ar}}}V_{\mathrm{a}}\right)\mathrm{e}^{-K_{\mathrm{a}}\Delta t}\right] \\ & - \left(\frac{M_{\mathrm{w}(t-1)}}{V_{\mathrm{w}}}V_{\mathrm{wc}}n_{\mathrm{w}(t-1)}\right) + \left[\left(\frac{n_{\mathrm{w}(t-1)}M_{\mathrm{tot(t0)}}}{V_{\mathrm{w}}}V_{\mathrm{wc}}\right)\mathrm{e}^{-K_{\mathrm{w}}\Delta t}\right] \\ & - \frac{M_{\mathrm{b}(t-1)}}{V_{\mathrm{b}}}V_{\mathrm{b1}}n_{\mathrm{b}(t-1)} \end{aligned}$$

where: $M_{\mathrm{a}(t-1)}$ = moles in air at time $t-1$ (mol); $M_{\mathrm{w}(t-1)}$ = moles in water at time $t-1$ (mol); $M_{\mathrm{b}(t-1)}$ = moles in biota at time $t-1$ (mol); K_{a} = overall degradation rate constant in air (days^{-1}); K_{w} = overall degradation rate constant in water (days^{-1}); K_{b} = overall degradation rate constant in biota (days^{-1}); Δt = time interval between t and $t-1$ (days); V_{ar} = volume of air in the room (m^3); V_{wc} = volume of water during the water changes (m^3); $n_{\mathrm{w}(t-1)}$ = number of water changes at time $t-1$; $n_{\mathrm{b}(t-1)}$ = number of animals removed at time $t-1$; and V_{b1} = volume of one animal (m^3).

3. Results and discussion

3.1 *Analytical results for water and biota*

The analytical results obtained for water and biota samples collected in the course of the experiments are shown graphically in figure 1. Data related to exposure water show a very rapid initial decreasing trend until the second day, followed by a more stable prolonged phase. In this second phase, the concentrations tend to stabilize or slightly rise until the end of the experiment, as would be expected by the constant additions of new compound related to daily water renewal. This behaviour was clear especially with the higher TPT exposure concentration but could also be observed for the two other concentrations tested. These trends reveal the typical behaviour of the compounds in the actual experimental system employed. The mean values of the TPT concentrations in water during the exposure test as well as the TPT concentrations a few hours after the start of the experiment (i.e. after the initial introduction of the molecule in the system) were surprisingly low. Time-weighted mean concentrations and standard deviations can be calculated after the initial rapid decrease (3–28 day interval). These gave 5.5 ± 3.1, 14 ± 6.3, and $33 \pm 11\ \mathrm{ng\,L^{-1}}$ for the nominal concentrations of 100, 225, and $500\ \mathrm{ng\,L^{-1}}$ of TPT-Cl, respectively. These actual TPT concentrations will have to be duly taken into account for further interpretation of biological effects. The measured concentrations were much lower than the nominal concentrations. The differences between nominal and analytical concentrations are almost comparable for the three TPT exposure levels. Measured concentrations were around 15–20 times less than the

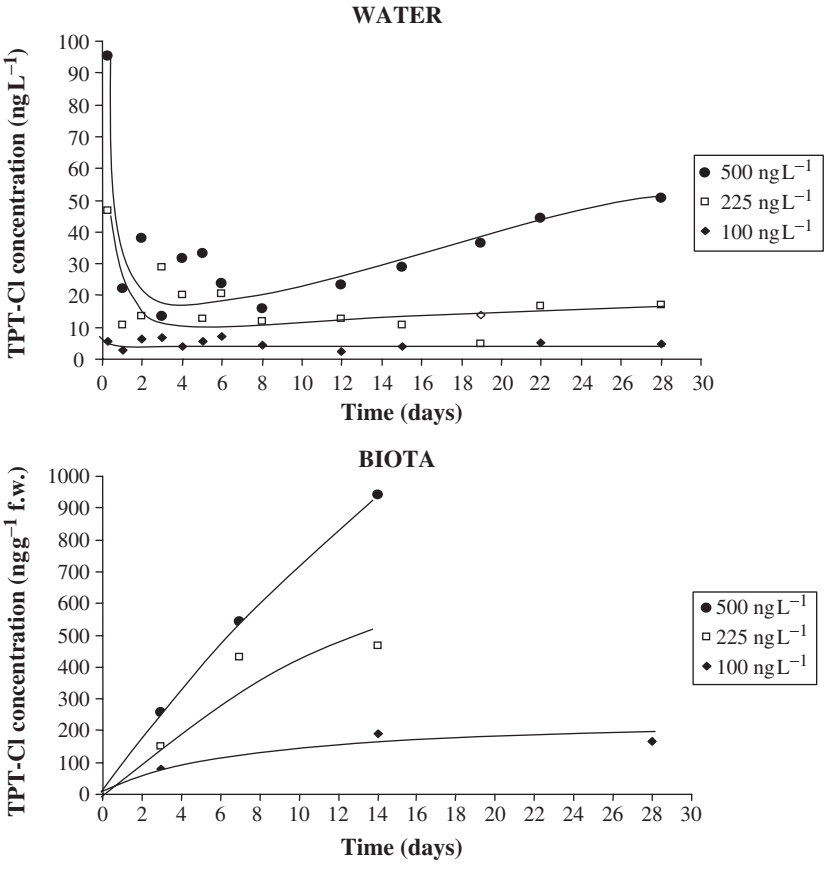

Figure 1. TPT-Cl measured concentrations in water and biota for the nominal concentration of 100, 225, and 500 ng L^{-1}.

nominal concentrations (mean factor of 16.7 ± 1.5). A factor of 15–20 between nominal and analytical concentrations means that loss processes account for 92–95% of the added compound.

Taking into account the physico-chemical properties of the compound (see table 1), a consistent transfer of TPT from water to biota can be expected. In contrast, degradation processes should play only a secondary role in air and water. TPT is considered to be resistant to photodegradation and hydrolysis [19], even if these do occur [31]. In addition, the low light intensity and low temperature (15–16°C) maintained in the experimental aquaria indicate that the effectiveness of photodegradation is negligible. On the other hand, biotransformation can be more important, because it is known that triphenyltins can be dealkylated (via di- and monophenyltin) to inorganic tin in the midgut gland of certain invertebrates [8] (e.g. crustaceans and molluscs). The same products have been observed in sea water, following microbial degradation [32, 33]. According to these data, in the present work both monophenyltin and diphenyltin were detected and quantified at high levels (mean values of 230 and 24% dealkylated species/triphenyltin, in molar basis, for water and biota, respectively). Current research is under way,

exploring this point in detail, and the complete data are still to be published. Dealkylated products are mainly present in water, while the parent compound is prevalent in biota, because of an efficient bioconcentration process. High BCF values are reported in the literature, namely in molluscs [34] (BCF on a fresh weight of 3.6×10^4 and 4.3×10^4, respectively, for *Mytilus edulis* and *Mytilus graynus*). Also in this work, the TPT concentrations found in the organisms clearly show the existence of a relevant bioconcentration process. The TPT concentrations measured in the tissues of *A. mediterranea*, (figure 1) as $\mathrm{ng\,g^{-1}}$ fresh weight, are quite high and seem to increase until the 14th day, to reach an almost steady-state condition. For a BCF evaluation, it is important to remember that BCF should be calculated when the saturation level (intake and release rates are equal) is reached, after a period of constant exposure concentration, indicatively 7 days [35]. In the present work, although there is no analytical evidence that the experimental system has actually reached the effective saturation state, a BCF value is calculated for *A. mediterranea*. The time of the exposure experiments, the rather constant TPT concentrations in water during the selected 3–28 interval, and the consistent results obtained with the three concentrations tested allow make it possible to assess a BCF value for the test species. On the basis of the available data, three BCF values, volume/volume (v/v) on a fresh-weight basis, can be calculated: 3.3×10^4, 4.4×10^4, and 2.8×10^4, for the nominal concentrations of 100, 225, and $500\,\mathrm{ng\,L^{-1}}$, respectively. From these values a mean BCF (V/V) on a fresh-weight basis of $3.5 \times 10^4 \pm 0.8 \times 10^4$ (standard deviation) can be derived along with the corresponding mean BCF (V/V) on a dry-weight basis of $1.4 \times 10^5 \pm 0.3 \times 10^5$ (standard deviation). The above reported BCF values were calculated, taking into account the time-weighted mean TPT concentrations in water between the 14th and the 28th day and the mean TPT concentrations in tissues, if available, in the same interval. BCF values (V/V) on fresh and dry weights were obtained with the calculated mean water content of *A. mediterranea* (56%) and a density of 1.2 and $1.6\,\mathrm{g\,mL^{-1}}$ for the fresh and dry matter, respectively. However, owing to the limited number of samples used for analytical determination of TPT levels in the organisms, these BCF values should be considered with caution. On the other hand, our exposure experiments were originally addressed not to bioconcentration evaluation but to a detailed study of biological effects, which meant that most of the tissue samples had to be reserved for this purpose.

The BCF assessed in this work for *A. mediterranea* matches the value reported for *Mytilus edulis* [34] (BCF on a fresh weight of 3.6×10^4) and is very similar to values found for *Mytilus graynus* [34] (BCF on fresh weight of 4.3×10^4) and slightly higher than those reported for the freshwater mollusc, *Dreissena polymorpha* [12] (BCF on a dry-weight basis of 2.4×10^4 to 6.9×10^4). The BCF for *A. mediterranea*, like that reported for the other invertebrates, generally appears to be more than one order of magnitude higher than those known for fish [35–37] (BCF on a fresh-weight basis of 1.1×10^3 to 0.53×10^3 for *Lebistes reticulatus*; BCF on a dry-weight basis of 2.3×10^3 in *Gnathopogon caerulescens* and of 3.1×10^3 to 4.1×10^3 for different marine fish species). The results of the present work confirm the difference between fish and invertebrates regarding the bioaccumulation capacity. This difference can be related to a diverse biotransformation efficiency of organotin compounds among the different taxonomic groups [38].

3.2 *Model development and validation*

The application of the level II fugacity model, fitted to the present experimental system, makes it possible to describe the daily concentration trends in air, water, and biota. Predicted data for these three compartments are reported in the graphs of figure 2 together with measured data, when available. Predicted trends in water for the three concentrations tested show a very rapid initial decrease, mainly due to the transfer

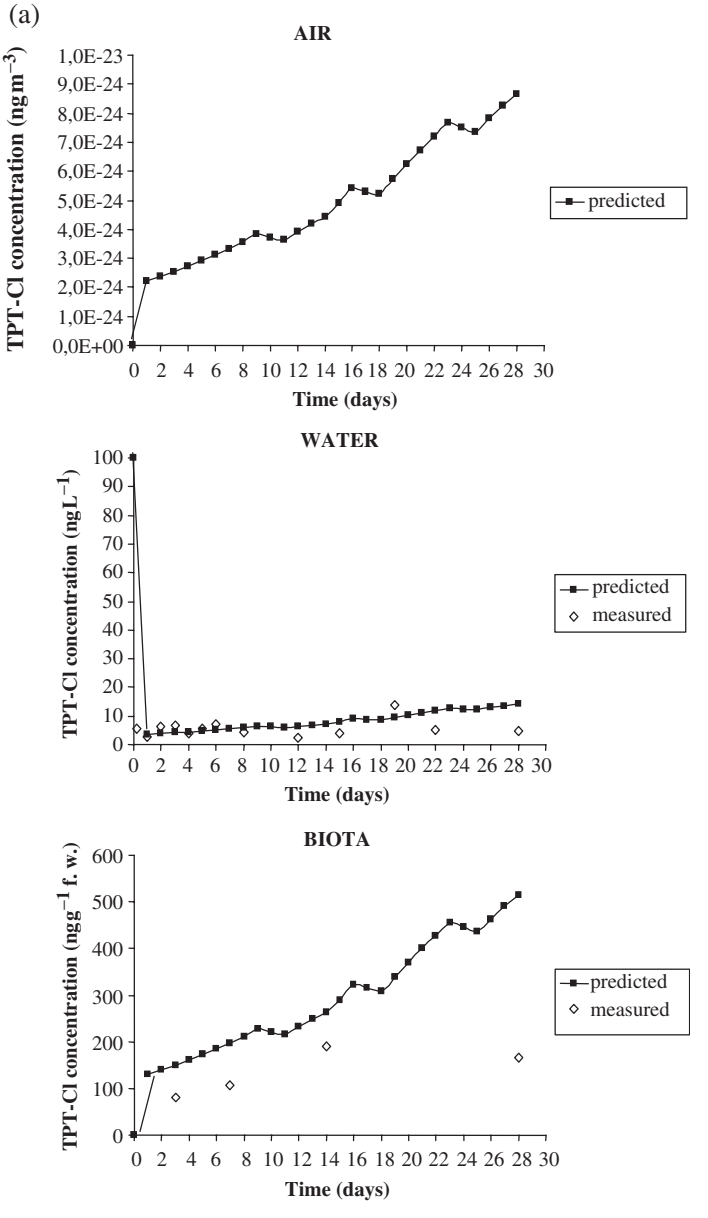

Figure 2. Predicted vs. measured, when present, TPT-Cl concentrations in air, and biota for the nominal concentration of: (a) 100 ng L^{-1}, (b) 225 ng L^{-1} and (c) 500 ng L^{-1}.

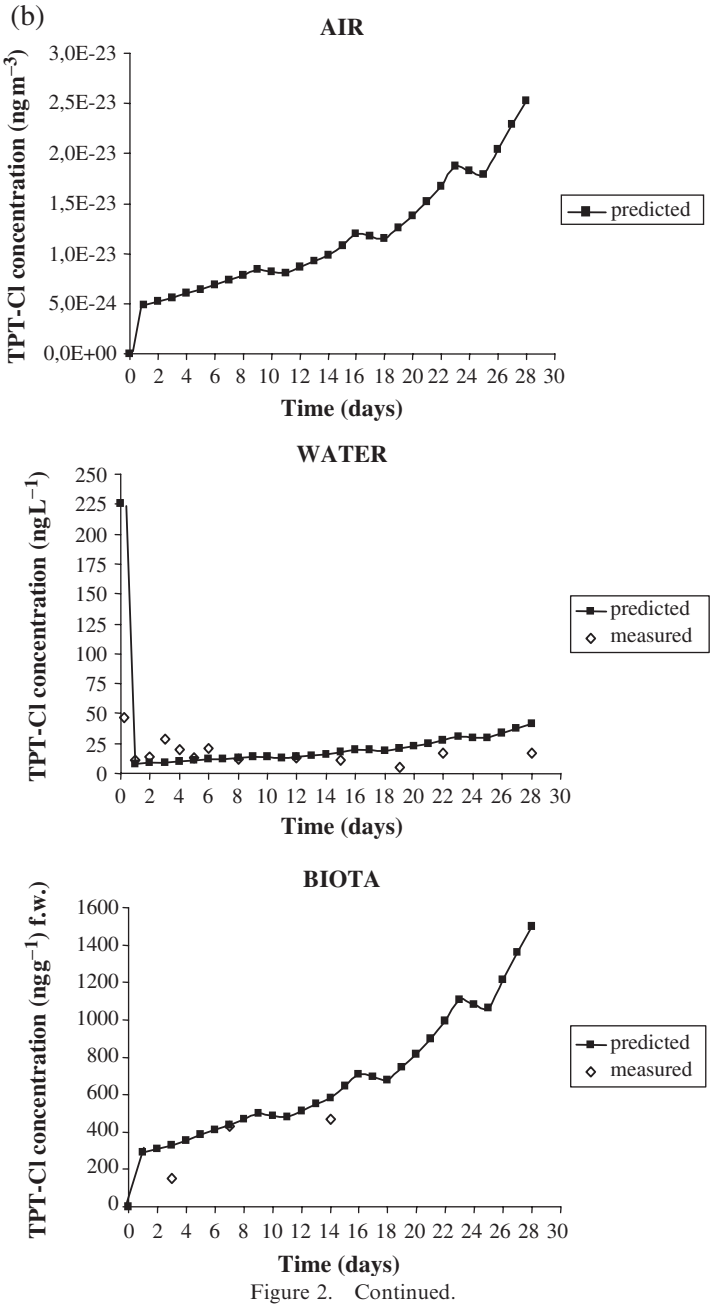

Figure 2. Continued.

of the compound to the biota compartment (bioconcentration). Other TPT losses from water, such as volatilization and degradation, are minor processes. After this first phase, predicted concentrations exhibit a slow but quite constant increase, because of the constant additions of new compounds by means of water renewal. These additions exceed the considered loss processes (reaction in the three compartments and advection, out of the system, by air and water during the water renewals).

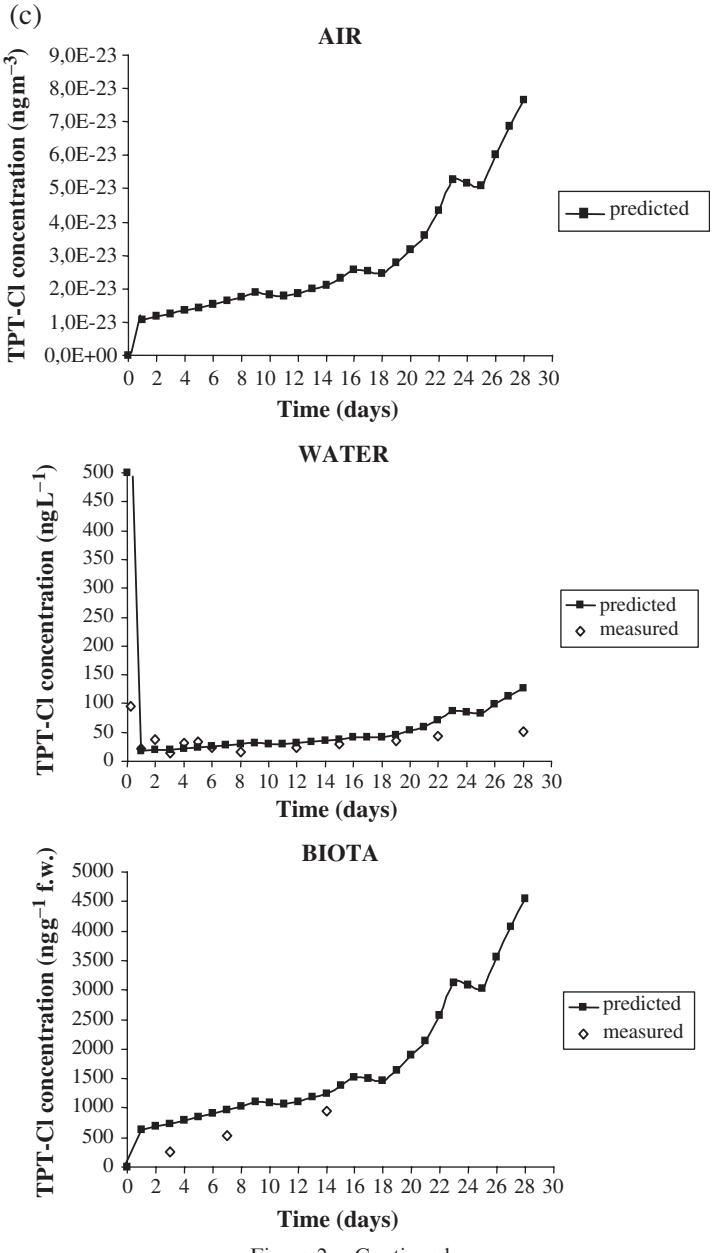

Figure 2. Continued.

Predicted data in air and biota show an initial rapid increase because of the first partitioning of the compound dissolved in water at the beginning of the experiment. After this fast step, the increase slows following the predicted water levels. Since the compound concentration increases in water, there is an increase in the net transfer to the air compartment over the water layer and into the biota. The predicted concentrations in water and biota match the measured concentrations, with particular reference to the drop of the nominal concentrations after one day. At least for the first 15 days,

Table 3. Loss (output) and addition (input) processes from water, quantified by the model as number of moles or percentage of moles for the TPT-Cl nominal exposure of 500 ng L^{-1}.

Process	Compartment	Moles	Percentage of moles
Reaction	Air	1.4×10^{-19}	6.4×10^{-11}
	Water	1.2×10^{-9}	0.58
	Biota	0.3×10^{-7}	14.69
Advection out	Air	1.5×10^{-17}	6.8×10^{-9}
	Water	0.1×10^{-7}	5.59
Transfer	Biota	1.7×10^{-7}	79.14
Total output		2.1×10^{-7}	100
advection in	Air	2.3×10^{-21}	1.7×10^{-12}
	Water	2.1×10^{-7}	100
Total input		2.1×10^{-7}	100

the measured concentrations in water reach values that are exactly in between the predicted values. Then, they do not appear to increase as much as the predicted concentrations, thus revealing that the loss processes in the experimental system are more efficient than those considered in the model. The trends of the predicted concentration in the biota show an overestimation by a factor of about 2 (mean factors of 2.1, 1.5, and 2.0 for nominal concentrations of 100, 225, and 500 ng L^{-1}, respectively) with respect to the measured actual concentrations. A real difficulty for the evaluation or appraisal of the environmental fate of a chemical is the definition of the overall degradation rates in each compartment, since they are highly dependent on the compound itself, the environmental conditions and the specific organisms present in the system, including the selected animal test species and the micro-organism flora ubiquitous in each biological system. In the present study, the increase in water concentration observed during the experiments is lower than the increase calculated by the model and can be likely explained by reaction rates higher than those selected in the model (table 1). Nevertheless, the predicted *versus* measured concentrations, differing by a factor of 2 both in water and in biota, suggest that the developed model has a very good predictive capability. Furthermore, this confirms the validity of the fugacity approach at the microecosystem level. For the three concentrations used, the observed decrease in the TPT concentrations in water seems to be rather compatible with the bioconcentration process. Therefore, the primary loss from water could be due to bioconcentration, accounting for around 80% of the total loss processes, as emphasized by the mass balance reported in table 3. A secondary loss mechanism is the reaction in the biota, explaining 15% of total compound loss. Advection by water constitutes a minor amount of loss for TPT. The other reactions (in air and water) and advection processes (via air) play only a minor role in compound loss (less than 1% as a whole). Concerning the input processes of TPT, advection via water turns out to be the only effective pathway, because contamination via air appears to be negligible, taking into account the very low vapour pressure and Henry's constant for this compound.

4. Conclusion

Two different results have been achieved in this work: (1) the measurement of an experimental BCF value for *A. mediterranea* and (2) the validation

of a simple model for quantitatively predicting compound loss processes in aquatic ecotoxicological texts.

On the basis of the analytical data in water and biota, a BCF value (v/v) on a fresh-weight base of $3.5 \times 10^4 \pm 0.8 \times 10^4$ (standard deviation) can be calculated for *A. mediterranea*. With regard to the second point, on the basis of the good agreement found between the predicted and measured concentrations of TPT in both water and biota for the three tested concentrations, the assumptions of our model appear to be valid. This simple first attempt provides a good test of the potential of the model in the applied field of ecotoxicology. It explains quite clearly the main behaviour of TPT in both water and biota during the exposure period, suggesting that the low measured concentrations in water are mainly due to bioconcentration process, whereas advection and reaction are only of secondary importance. Transformation processes must be carefully considered in long-term ecotoxicological tests [2], and in this case also, they partly explain the differences between nominal and measured concentrations. Therefore, this simple model can be considered as a useful tool on a wider basis for the planning phase of any ecotoxicological test, in particular for the evaluation of the relative importance of the main loss processes in the course of the experiments. It also provides an interesting approach for understanding the compound behaviour at the microecosystem scale. In this view, the developed model can be of great help in establishing the experimental conditions of the specific test, such as the volume of water in the aquarium, the animal biomass, and the water renewals, in relation to the molecule and the organism tested.

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