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The acute toxicity of thallium to freshwater organisms: Implications for risk assessment

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HIGHLIGHTS

- The toxicity of thallium to four freshwater organisms has been studied.
- The microalga, *Pseudokirchneriella subcapitata*, was most sensitive to Tl.
- Tl toxicity to *Daphnia* was greater in tap water than in artificial water.
- Various sub-lethal effects were observed in early-life stage *Danio rerio*.
- A PNEC for Tl of 0.087 μg l^{-1} is proposed.

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ABSTRACT

The acute toxicity of Tl(I) to the microalga, *Pseudokirchneriella subcapitata*, the planktonic crustaceans, *Daphnia magna* and *Daphnia pulex*, and early-life stage of the zebrafish, *Danio rerio*, has been studied according to OECD protocols. Toxicological end-point concentrations for the microalga ranged from 17 μg l^{-1} for a 72 h E_yC_{25} (yield inhibition) to 80 μg l^{-1} for a 72 h E_rC_{50} (growth inhibition). *Daphnia* were less sensitive to Tl, with 48 h EC_{50}s of about 1000 μg l^{-1} and 1200 μg l^{-1} for *D. magna* and *D. pulex*, respectively; however, end-point concentrations were reduced considerably (to about 510 μg l^{-1} and 730 μg l^{-1}, respectively) when experiments were repeated in dechlorinated Plymouth tap water (rather than OECD medium). The 96 h LC_{50} for *D. rerio* was 870 μg l^{-1} but a variety of sub-lethal effects, including enlargement of yolk sac and reduction in heart beat rate, were observed when larvae were exposed to lower concentrations. Based on these results, a predicted no effect concentration (PNEC) for Tl in freshwaters of 0.087 μg l^{-1} is proposed. The PNEC is an order of magnitude lower than the only (Canadian) water quality guideline for Tl that appears to exist, and is lower than Tl concentrations reported in freshwaters impacted by historical or contemporary metal mining. Our results are also consistent with previous studies that employ different organisms and end-points in that Tl toxicity is dependent on the concentration of K^+, the biogeochemical analogue of Tl^+. Accordingly, regulation of Tl in the freshwater environment should factor in the relative abundance of K.

1. Introduction

Thallium is a non-essential, highly toxic heavy metal. Forming salts with both monovalent [thallous (I)] and trivalent [thallic (III)] oxidation states, it has a variety of properties and characteristics. Thus, Tl is very similar to Pb in terms of gravity, hardness, appearance, melting point and electrical conductivity (Galvan-Arzate and Santamaria, 1998). However, Tl^+ shares many characteristics with the alkali metals and is considered to be biogeochemically very similar to the potassium ion, K^+ (Kaplan and Mattigod, 1998; Hassler et al., 2007).

Thallium is a relatively rare metal with an average concentration in the lithosphere of 1 μg g^{-1} (Smith and Carson, 1977). Although several Tl-bearing minerals exist, the metal is encountered mainly in minerals of potassium, such as alkali feldspars and micas, in coal, and in sulphides. Consequently, Tl is usually recovered for use in various specialist industries as a byproduct from flue dusts and residues resulting from the smelting and refining of sulphidic ores. As a contaminant, Tl enters the environment largely from the burning of coal and from metal mining and smelting (Peter and Viraraghavan, 2005).

Although Tl is more acutely toxic to mammals than Cu, Hg, Cd, Pb and Zn (Cheam, 2001), there are relatively few studies that address its accumulation and effects in aquatic organisms. This is, perhaps, surprising since in many environments, and in particular those impacted by historical and contemporary metal mining, concentrations of aqueous

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Tl often exceed concentrations of other heavy metals, like Cd and Pb, whose toxicities are much better defined (Tatsi and Turner, 2014). An examination of the literature reveals that, in fresh water and according to a variety of end points in a range of organisms, Tl(I) toxicity ranges from a few μg l^{-1} to a few mg l^{-1} (Zitko et al., 1975; Kwan and Smith, 1988; Borgmann et al., 1998; Pickard et al., 2001; Lin et al., 2005; Hassler et al., 2007; Rickwood et al., 2015). Toxic concentrations appear to be reduced in the presence of competitive ions, and in particular K^+, the biogeochemical analogue of Tl^+, but not by natural or artificial complexants, such as humic acids and EDTA (Zitko et al., 1975; Borgmann et al., 1998; Lustigman et al., 2000). Only one study appears to have completely isolated Tl(III) and addressed its toxicity (Ralph and Twiss, 2002); thus, while Tl^{3+} was found to be considerably more toxic than Tl^+ to the unicellular alga, *Chlorella*, the relative abundance of the thallic ion is predicted to be vanishingly low in most natural waters.

The Canadian Water Quality Guideline for total dissolved Tl is 0.8 μg l^{-1} (CCME, 1999). This guideline was derived from the chronic (28 d) toxicity of Tl(I) to the macrophyte, *Lemna minor* (Brown and Rattigan, 1979), the most sensitive aquatic organism and end-point reported at the time, coupled with a tenfold safety factor. Despite this quality standard, and the US recognising Tl as a priority pollutant, the metal is neither regulated in European waters nor considered as part of the Water Framework Directive (Commission of the European Communities, 2006).

The purpose of the present study was to assess the acute toxicity of Tl(I) to three key trophic species according to standardised OECD methods. To this end, we selected an algal growth test, the *Daphnia* immobilisation test and the fish, early-life test, and used end-points and timescales recognised for ecotoxicological hazard assessment. The results of the present investigation, together with those of previous studies, are used to re-evaluate the first tier of hazard assessment for Tl and propose refined water quality standards for the metal in the environment.

2. Materials and methods

Before being used in the experiments, all glassware was soaked in 5% HNO_3 for 24 h and subsequently rinsed in Millipore Milli-Q water (MQW; 18.2 MΩ · cm). Working stock solutions of Tl(I) were prepared by diluting different quantities of a 10,000 mg l^{-1} Aristar solution of Tl in 0.5 M HNO_3 (BDH Prolabo; CAS # 7440-28-0) in a series of 100 ml volumetric flasks using MQW. Test waters were prepared according to the appropriate OECD guidelines or by dechlorinating Plymouth tap water by bubbling air through 30 l contained in a darkened polyethylene tank overnight. Unless otherwise stated, all other reagents used in the experiments and for sample processing or preservation were purchased from Fisher Scientific or Sigma-Aldrich.

2.1. Algal growth inhibition test

The algal growth inhibition test was conducted using a commercially available kit (Algaltoxkit F™ by MicroBioTests Inc., Belgium). The kit employs the microalga, *Pseudokirchneriella subcapitata* (product code CCAP278/4), and the test was performed following the standard operational procedure provided by the manufacturer and in accordance with the OECD 201 guideline (OECD, 2006). Briefly, tests were conducted at 23 °C in controlled temperature incubators under uniform illumination of 10,000 lx and using the 25 ml cuvettes supplied with the kit. The growth medium was MQW amended with various salts, nutrients and EDTA and whose pH was adjusted to 8.1 by the addition of NaOH (and as monitored using a Hydrus 500 glass combination electrode). Cells were seeded at a density of 10,000 ml^{-1} by measuring the turbidity of the medium at an absorbance of 670 nm using a 7315 spectrophotometer (Jenway Ltd, UK) against a standard curve of cell density provided with the Algaltoxkit F™ kit. Growth rate was determined daily over a period of 72 h using the same technique.

The experiment was performed in triplicate and using different concentrations of Tl obtained by diluting appropriate quantities of the various working stock solutions in algal culturing medium. Specifically, following an initial range-finding test, the exposures were conducted between 40 μg l^{-1} and 400 μg l^{-1} and included a Tl-free control. The pH was monitored in the test cells at the beginning and end of the experiment and 5 ml water samples were pipetted from each cuvette at the termination of the exposures into 10 ml Sterilin tubes and acidified with 100 μl of HNO_3 pending analysis of Tl, Na, K and Ca by ICP (see below).

2.2. Daphnia acute immobilisation test

Given the paucity of acute toxicity data for Tl on invertebrates, experiments were conducted on two species of cladoceran, *D. magna* and *D. pulex*, according to OECD guideline 202 (OECD, 2004). Stocks of *D. magna* and *D. pulex* were purchased from Sciento (Manchester, UK) and were maintained in separate, 10 l aquaria containing reconstituted OECD test water (MQW water amended with various salts) at 21 °C and under a 12 h fluorescent light:12 h dark photoperiod for at least 2 weeks prior to use. Neonates were exposed in triplicate (with 30 animals per treatment) and for 48 h to Tl(I) concentrations ranging from 60 to 1200 μg l^{-1} (based on results of a range-finding test, data not shown) and to a Tl-free control in 40 ml of OECD water in a series of 100 ml plastic Galli pots under the culture conditions described above. Dissolved oxygen and pH were measured using a HACH HZ40d multi-meter with a glass combination electrode at the beginning and end of the exposures for the lowest and highest Tl concentrations employed. Five millilitre water samples were taken from each exposure vessel as above at the beginning and termination of all treatments for subsequent analysis of Tl and alkali metals.

Experiments were repeated using dechlorinated Plymouth tap water in order to establish whether Tl in natural water elicited a different response to Tl in the OECD medium. Here, animals were acclimatised in tap water and exposed to tap water amended with Tl under otherwise identical conditions to those described above.

2.3. Fish, early-life stage toxicity test

The early-life stage fish test was carried out following OECD 210 guidelines (OECD, 1992). Stocks of adult male and female zebrafish (*Danio rerio*) were placed together in a 20 l, aerated, flow-through glass tank containing dechlorinated tap water at 28 ± 2 °C and under a photoperiod of 14 h fluorescent light:10 h dark. Resulting embryos were collected approximately 30–60 min after spawning and carefully graded using a Kyowa Optical microscope (Model SDZ-PL; zoom HWF 10×). Viable embryos that were at the 8-cell stage or beyond (n = 360) were randomly selected for the exposures. Embryos were exposed in triplicate (with 20 embryos per treatment) and for 144 h to Tl(I) concentrations ranging from 50 to 800 μg l^{-1} (established from a range-finding test, data not shown) and to a Tl-free control in 300 ml of dechlorinated tap water in a series of 400 ml Pyrex beakers under the culture conditions described above. A semi-static exposure method was adopted with 2/3 of the water changed every 24 h with appropriate re-dosing (i.e. 2/3 of the nominal dose). The number of dead and living embryos and/or larvae was counted by visual inspection or by optical microscope every 24 h and before each water change. The time of hatching and any abnormal behaviour or appearance were also noted. Water quality measurements were taken each day prior to water changes using a HACH HZ40d multi-meter. Five millilitre water samples for subsequent analysis of Tl and alkali metals were taken from each exposure vessel at the beginning, after every water change and at the end of all treatments.

Table 1
Characteristics of the different media used in the experiments. For pH, DO (dissolved oxygen) and ionic concentrations, the grand mean and one standard deviation are given (note that DO was not measured in the algal exposures).

Organism	Medium	Temp. (°C)	pH	DO (mg l^{-1})	$[Na^+]$ (mg l^{-1})	$[K^+]$ (mg l^{-1})	$[Ca^{2+}]$ (mg l^{-1})	$[Mg^{2+}]$ (mg l^{-1})
P. subcapitata	OECD 201	23	7.90 ± 0.04	–	30.2 ± 0.9	1.00 ± 0.03	8.9 ± 0.1	5.0 ± 0.1
D. magna/D. pulex	OECD 202	21	7.70 ± 0.19	8.23 ± 0.05	17.6 ± 0.4	3.00 ± 0.08	71.0 ± 0.2	11.0 ± 0.1
	Tap water	21	7.60 ± 0.23	8.12 ± 0.05	11.1 ± 0.3	1.45 ± 0.08	25.0 ± 0.3	2.1 ± 0.3
D. rerio	Tap water	28	7.50 ± 0.16	7.18 ± 0.07	6.2 ± 0.1	1.25 ± 0.05	16.5 ± 0.1	6.2 ± 0.7

Morphometrics were measured on surviving larvae at the end of the experiment. Briefly, larvae were euthanized in 200 mg l^{-1} of buffered MS222 and then fixed in formalin. Larvae ($n = 4$ per beaker, $n = 12$ per treatment) were photographed (at 1.6× and 4× magnification) using a Lumenera Infinity 2 digital camera attached to a Meiji binocular microscope and subsequent size measurements were undertaken manually using Image J 1.46r software. The length of the larvae and the width of the spinal cord muscle block were measured as growth indicators while the length (L, mm) and height (H, mm) of the yolk sac were measured to identify any changes in sac volume (V, $mm^3 = 6/\pi LH^2$) and as indicators of oedema (Velasco-Santamaría et al., 2011).

Because of the possibility that Tl toxicity may be mediated by interference with K^+ homeostasis, the heart beat rates of the fish were also measured during the experiment (K^+ is essential for heart contractility). Measurements were conducted after an exposure period of 96 h on surviving larvae randomly selected from each beaker dosed with the highest concentration of Tl and from each beaker containing no added Tl. Individuals were placed in a small volume of dechlorinated tap water under a binocular microscope and heart beat rate was determined manually and in triplicate by recording the number of beats over 30 s; larvae were then placed back into their beakers for the remaining period of the exposures.

2.4. Metal analysis

Thallium in water samples from the experiments and in a certified reference material (Drinking Water 904020, Charleston, SC; Tl = 10 μg l^{-1}) were analysed using a Thermo Scientific X Series 2 II bench top quadrupole inductively coupled plasma-mass spectrometer (ICP-MS) with a collision cell according to operating conditions given in Turner et al. (2010). The instrument was calibrated using a blank and five standards in the range 10 to 1000 μg l^{-1} (prepared by serial dilution of the Prolabo Aristar standard in 0.3 M HNO_3) and internal standardisation was achieved by the addition of 100 μg l^{-1} of ^{193}Ir to all samples and standards. The relative standard deviation of replicate measurements of Tl in all samples was generally less than 10% and accuracy, based on analyses of the reference material, was better than 90%. Limit of detection, based on 3σ of multiple measurements of the lowest standard, was 6 ng l^{-1}.

Ions essential for fish and invertebrate health and that may compete with Tl^+ at the cellular level (Na^+, K^+, Ca^{2+}, Mg^{2+}) were analysed as their respective elements by ICP-optical emission spectrometry using a Varian 725-ES according to operating conditions outlined by Jessop and Turner (2011). The instrument was calibrated using five mixed standards and a blank prepared by serial dilution of Spectrosol plasma emission standards in 0.3 M HNO_3.

2.5. Data treatment and statistical analysis

The percentage inhibition of both specific growth rate (E_rC_x, based on the logarithmic increase of biomass during the test period) and yield (E_yC_x, based on the biomass at the end of the test minus the starting biomass) were calculated after treatment of data according to OECD guideline 201 (OECD, 2006). The effect concentrations to 50% and 25% of the population of *Daphnia* at 48 h (EC_{50} and EC_{25}, respectively) were estimated by fitting a sigmoid curve to the data using Sigma Plot *v*12.5. Likewise, and regarding zebrafish, the lethal concentrations to 50% and 25% of the population at a specific time (LC_{50} and LC_{25}, respectively) and the lethal time to 50% of the population for a given concentration (LT_{50}) were estimated by sigmoid curve-fitting.

Following tests for unequal variance (Bartlett's test), one way analysis of variance (ANOVA) was used to determine the statistical significance of differences in water quality parameters between tests and among replicates, and differences in zebrafish morphometric data arising from the different treatments. All statistical analyses were performed using StatGraphics Centurion XVI.I and used a rejection level criterion of $p < 0.05$.

3. Results

3.1. Water characteristics and confirmation of Tl exposure

The physico-chemical characteristics of the waters used in the experiments are shown in Table 1. For pH, dissolved oxygen and concentrations of the alkali metal ions, the grand mean and standard deviation arising from replicate measurements taken at multiple times and/or multiple (Tl) exposure concentrations are shown. For a given experiment, no statistically significant differences in water characteristics were observed during the time course or between different exposures. Note, however, that the composition of tap water (and in particular concentrations of Na and Mg) was different on the two occasions used for the exposures. Note also that the concentration of dissolved organic carbon in tap water, although not measured directly, averages about 0.8 mg l^{-1} for the region according to local water authority data.

In all cases, concentrations of Tl measured throughout the experiments did not deviate substantially from nominal (added) concentrations. This is exemplified by plots of measured versus nominal

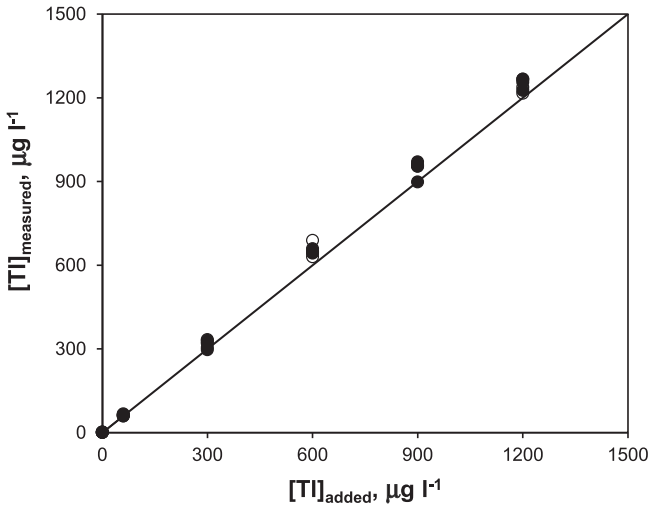

Fig. 1. Concentrations of measured Tl versus concentrations of added Tl at the beginning (○) and end (48 h; ●) of the exposures to *D. magna* in OECD water. Note, the solid line represents unit slope.

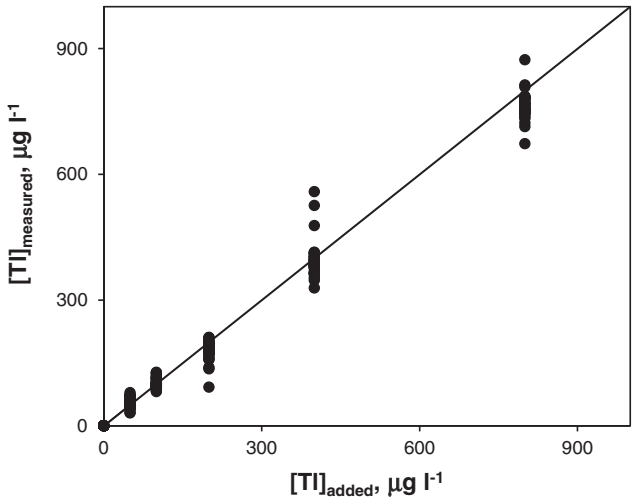

Fig. 2. Concentrations of measured Tl versus concentrations of added Tl at the beginning, at each water change and at the end (144 h) of the exposures to *D. rerio* in Plymouth tap water. Note, the solid line represents unit slope.

concentrations in the experiment in which *D. magna* were exposed to a single addition of Tl in OECD medium (Fig. 1) and *D. rerio* were exposed to Tl added to dechlorinated tap water that was replenished on a daily basis (Fig. 2). The results indicate that, during the experiments, Tl was not subject to significant inputs from (cross) contamination or to significant loss through, for example, precipitation or instantaneous or progressive adsorption to container surfaces.

3.2. Algal growth inhibition test

Results of the growth inhibition test in which *P. subcapitata* was exposed to variable Tl(I) concentrations in OECD medium are shown in terms of algal cell density in Fig. 3, and estimated toxic concentrations for the different end-points are shown in Table 2. The biomass increased exponentially by a factor of >16 in the controls, in agreement with the validation conditions of the test guidelines, and Tl reduced algal density by more than 50% at a concentration of 40 µg l^{-1} and by more than 95% at concentrations at and above 128 µg l^{-1}; complete inhibition of growth rate occurred at the two highest concentrations of Tl employed.

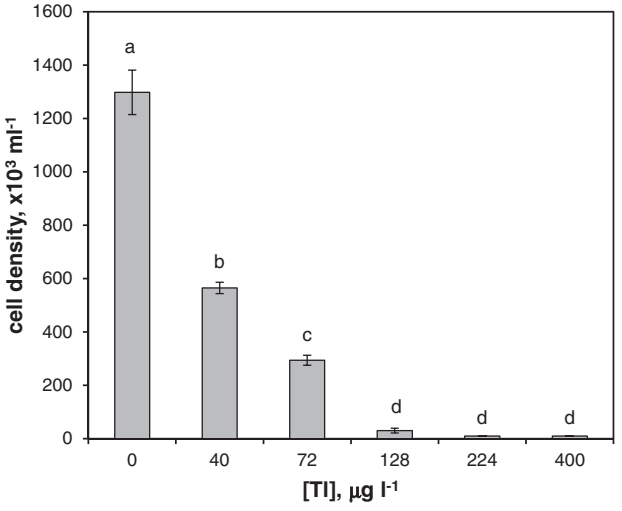

Fig. 3. Cell density of *P. subcapitata* as a function of nominal Tl concentration and as determined at the end of the 72 h exposure. Errors denote one standard deviation about the mean and different letters denote significant differences among treatments (ANOVA, $p < 0.05$).

Table 2
Estimated toxic concentrations of Tl(I) to the four organisms and for the different end-points.

Organism	Medium	End-point	[Tl(I)], µg l^{-1}
P. subcapitata	OECD 201	72 h E_rC_{50}	87
		72 h E_yC_{50}	33
		72 h E_rC_{25}	40
		72 h E_yC_{25}	17
D. magna	OECD 202	48 h EC_{50}	1180
		48 h EC_{25}	750
	Tap water	48 h EC_{50}	510
		48 h EC_{25}	350
D. pulex	OECD 202	48 h EC_{50}	980
		48 h EC_{25}	740
	Tap water	48 h EC_{50}	725
		48 h EC_{25}	580
D. rerio	Tap water	96 h LC_{50}	870
		144 h LC_{50}	290
		144 h LC_{25}	160

From the inhibition results, values for 72 h E_rC_{50} and E_yC_{50} were estimated to be 87 µg l^{-1} and 33 µg l^{-1}, respectively, while values for 72 h E_rC_{25} and E_yC_{25} were estimated to be around 40 µg l^{-1} and 17 µg l^{-1}, respectively. Repeating the experiment using concentrations of Tl in the range 10 to 100 µg l^{-1} allowed us to establish a NOEC of less than 10 µg l^{-1}.

3.3. Daphnia immobilisation test

The effects of increasing Tl(I) concentration on the immobility of the cladocerans, *D. magna* and *D. pulex*, are shown in Fig. 4, and estimated toxic concentrations for the various end-points are shown in Table 2. Immobility was 0% in all controls and increased with increasing Tl concentration in each case but at different rates. Regarding *D. magna*, estimated values of 48 h EC_{50} and EC_{25} in dechlorinated tap water were 510 µg l^{-1} and 350 µg l^{-1}, respectively. In OECD water, the respective values were 1180 µg l^{-1} and 750 µg l^{-1} and greater than the corresponding values in tap water; the NOEC was less than 60 µg l^{-1} in tap water and between 60 µg l^{-1} and 300 µg l^{-1} in the OECD medium. With respect to *D. pulex*, estimated values of 48 h EC_{50} and EC_{25} in tap water were 725 µg l^{-1} and 580 µg l^{-1}, respectively, while in OECD water the respective values were 980 µg l^{-1} and 740 µg l^{-1}; the NOEC was between 60 µg l^{-1} and 300 µg l^{-1} in both media.

3.4. Fish, early-life stage toxicity test

The results of the tests performed on *D. rerio* are shown in Fig. 5 and estimated toxic concentrations for the different end-points are presented in Table 2. Cumulative survival is shown on a daily basis and cumulative mortality is shown at the termination of the experiment (144 h). In the Tl-free control, survival remained reasonably constant at about 95% (5% mortality represents a typical background level), while in the presence of Tl(I) a concentration-dependent response was observed once embryos had hatched. The LC_{50} at 96 h was estimated to be 870 µg l^{-1} while the LC_{50} and LC_{25} at 144 h were about 290 µg l^{-1} and 160 µg l^{-1}, respectively. For the highest exposure concentration (800 µg l^{-1}), the time to attain 50% lethality (LT_{50}) was about 100 h (4.2 days), and the NOEC at the termination of the experiment was less than 50 µg l^{-1}.

To assess the sub-lethal effects of Tl on *D. rerio*, the total length of the larvae, width of the spinal cord muscle block and volume of the yolk sac were measured in animals surviving at the end of the exposures. Although there were no statistical differences in larval lengths among the treatments, yolk sac volume increased with increasing exposure concentration up to 400 µg l^{-1}, or the highest concentration at which larvae survived (Fig. 6a); successive increases in yolk sac volume were not, however, always significant. When exposed to Tl, spinal cord

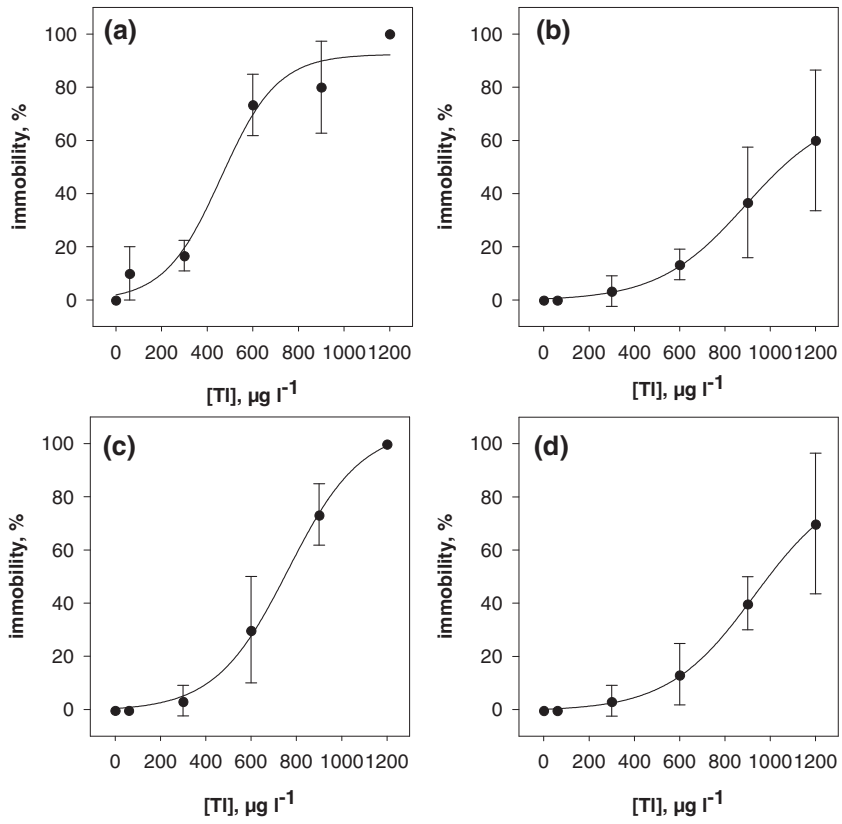

Fig. 4. Immobility of (a) *Daphnia magna* in Plymouth tap water ($r^2 = 0.93$, $p < 0.05$), (b) *D. magna* in OECD water ($r^2 = 0.78$, $p > 0.05$), (c) *D. pulex* in Plymouth tap water ($r^2 = 0.95$, $p < 0.05$) and (d) *D. pulex* in OECD water ($r^2 = 0.86$, $p < 0.05$) after 48 h exposure to different nominal concentrations of Tl. Errors denote one standard deviation about the mean.

muscle block width exhibited a significant increase relative to the control animals (Fig. 6b), although successive increases in Tl concentration did not result in any significant successive differences in width. After 96 h, larvae exposed to 800 µg l^{-1} appeared moribund and their heart beat rates (64 ± 4 per minute) were about 65% lower than rates for control larvae (216 ± 1 per minute).

In addition to the measurements above, imaging revealed enlarged pericardial areas, indicative of oedema, and a shortened snout (Fig. 6d), indicative of abnormal growth development. There were no concentration-dependent differences in hatching success of exposed animals, although a non-significant trend of increased time to hatch with increasing Tl concentration was noted (data not shown).

4. Discussion

4.1. Comparison of Tl toxicity with literature data

This study is one of relatively few to examine the toxicity of Tl to freshwater organisms. Specifically, it is the first to address the acute toxicity of Tl(I) to three trophic levels according to recognised (OECD) guidelines, the first to evaluate toxicity to two important test organisms (namely, *D. pulex* and *D. rerio*), and the first to examine sub-lethal effects on early-life stage fish. It is also one of very few studies in which Tl concentrations have been monitored throughout the exposures and controls.

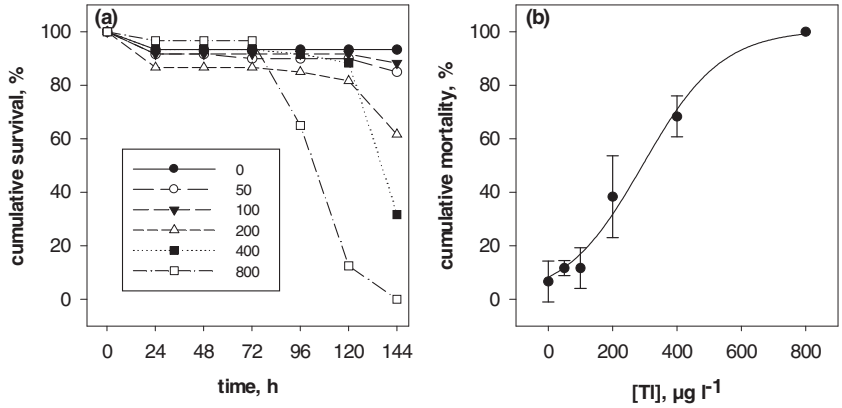

Fig. 5. (a) Cumulative survival of *Danio rerio* for each concentration of Tl (µg l^{-1}) and as a function of exposure time, and (b) mortality of *D. rerio* at 144 h exposure as a function of nominal Tl concentration ($r^2 = 0.98$; $p < 0.05$). Errors denote one standard deviation about one mean.

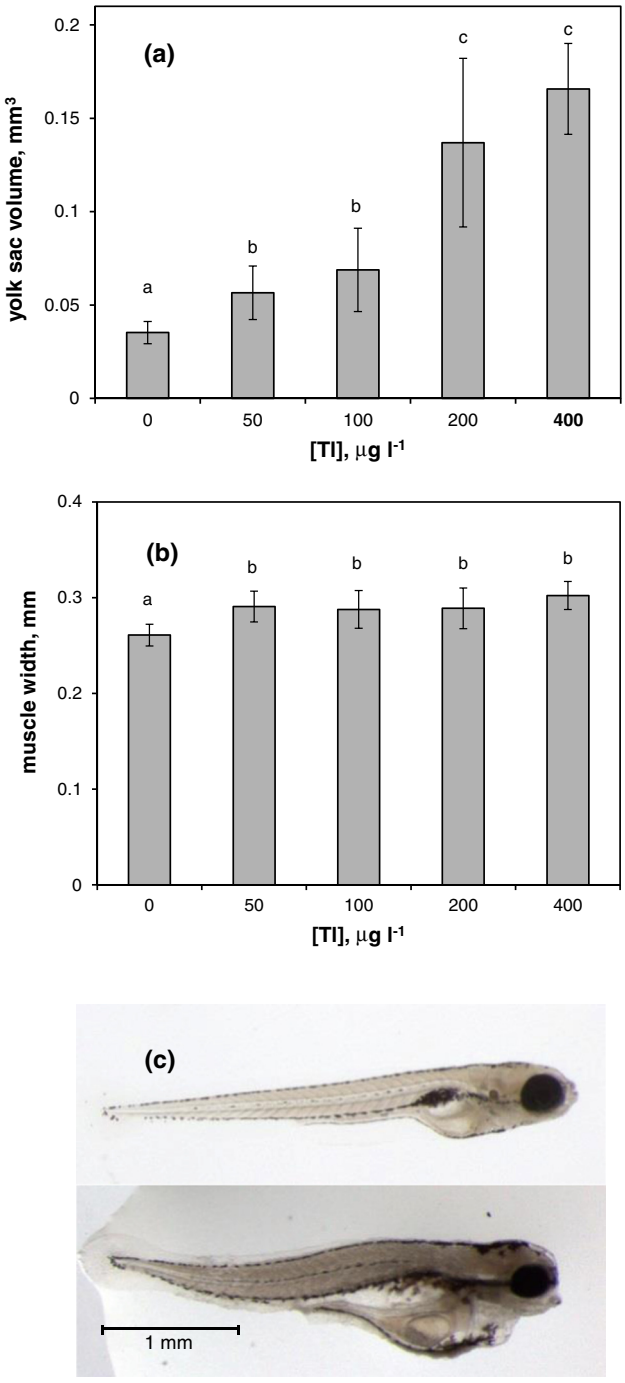

Fig. 6. Sub-lethal effects on *D. rerio* larvae exposed to Tl for 144 h: (a) Yolk sac volume and (b) spinal cord muscle width, where errors denote one standard deviation about the mean ($n = 12$) and different letters denote significant differences among treatments (ANOVA, $p < 0.05$), and (c) images of a control larva (above) and a larva exposed to 400 μg l^{-1} Tl (below).

To put the results of this study into context, Table 3 compares the toxicities of Tl(I) to freshwater primary producers, invertebrates and fish as reported in the literature and where comparable end-points (e.g. EC_x, LC_x, IC_x) are provided. Note that additional studies on Tl(I) toxicity in fresh waters, including those on bacteria and fungi or older studies where end-points are not always defined or quantified, have been reviewed by the EPA (1980) and Couture et al. (2011). Overall, our results are within the range of Tl concentrations reported in Table 3 that elicit a toxic response. More specifically, the lowest and highest concentrations we observed to be toxic to *P. subcapitata* (72 h $E_yC_{25} = 17$ μg l^{-1}; 72 h $E_rC_{50} = 87$ μg l^{-1}) are within an order of magnitude of independent results attained using the same microalga (72 h $IC_{25} = 90$ μg l^{-1} and 160 μg l^{-1}) and using *Chlorella* sp. (72 h $EC_{50} = 160$ μg l^{-1}). With respect to *Daphnia*, our range of toxic concentrations (48 h $EC_{25} = 350$ μg l^{-1}; 48 h $EC_{50} = 1180$ μg l^{-1}) is quantitatively similar to the concentration range reported in the literature for the survival of water fleas (168 h LC_{50} for *Ceriodaphnia dubia* = 370 μg l^{-1}; 48 h LC_{50} for *D. magna* = 2010 μg l^{-1}). Regarding early-life stage fish, our lowest and highest toxic concentrations (144 h $LC_{25} = 162$ μg l^{-1}; 96 h $LC_{50} = 870$ μg l^{-1}) compare with a 96 h LC_{50} and 168 h LC_{50} for *Pimephales promelas* (fathead minnow) of 860 μg l^{-1} and > 500 μg l^{-1}, respectively.

The end-point concentrations derived in the present study, coupled with a NOEC of less than 10 μg l^{-1} for *P. subcapitata*, would suggest that microalgae, at the base of the food web, are more sensitive to Tl than either invertebrates or fish. However, inspection of the results in Table 3 reveals toxicities of less than 10 μg l^{-1} for both the amphipod, *Hyalella azteca*, and the macroalga, *L. minor*, in addition to a 30 μg l^{-1} "incipient", 108 d, lethal toxicity to juvenile Atlantic salmon, *Salmo salar*, mentioned by Zitko et al. (1975). Our study has also demonstrated important sub-lethal effects to fish larvae exposed to a Tl concentration of 50 μg l^{-1} (enlargement of the yolk sac) and a significant impact on the heart, a vital organ, at the only exposure concentration tested (800 μg l^{-1}). The latter observation is of particular concern and could be related to or the result of yolk sac and pericardial oedema. Alternatively or additionally, the reduction in heart beat rate could be due to Tl^+ replacing the K^+ required for muscle contraction (Galvan-Arzate and Santamaria, 1998).

4.2. Thallium toxicity compared with other metal toxicities

In order to compare the fresh water acute toxicity of Tl(I) with the toxicities of other metals, results from the same tests and that employ identical end-points need to be considered. To this end, our end-point concentrations are compared with results from independent studies that have used the same tests but different metals in Fig. 7, while other studies that include Tl among a suite of other metals in specific toxicity tests are shown in Fig. 8. The comparisons reveal that, of the metals studied, and on a weight-concentration basis and neglecting speciation (and in particular, the relative abundance of the free ion), Tl is least toxic to microalgae (*P. subcapitata* and *Chlamydomonas reinhardtii*), most toxic to macrophytes (*L. minor* and *Elodea canadensis*, but not the clone of the former), and of intermediate toxicity to invertebrates (*D. magna* and *H. Azteca*) and early-life stage fish (*D. rerio*). On a molar basis the trends are similar, except that Tl is more toxic than Cd, Cu and Ni to the clone of *L. minor*, more toxic than Cu to *C. reinhardtii*, and more toxic than Cd and Zn to *P. subcapitata*.

4.3. Dependence of Tl toxicity on water chemistry

The physico-chemical characteristics of the aqueous medium usually have a significant impact on the toxicity of metals through both speciation and the abundance of competing ions. Equilibrium speciation calculations performed by Kaplan and Mattigod (1998) and Turner et al. (2010) suggest that, in natural fresh waters, the majority of Tl(I) occurs as Tl^+, and that in the absence of appreciable quantities of organic matter the relative abundance of the free ion is likely to approach 100%. With respect to artificial media, Tl(I) has a relatively low affinity for chelators that are often added, like EDTA (e.g. log *K* Tl-EDTA^{3-} = 7.3 cf log *K* Cu-EDTA^{2-} = 20.5; Couture et al., 2011). Thus, it would be reasonable to assume that in both OECD media and Plymouth tap water, the concentrations of Tl that are toxic in the present study apply to both total metal and the free ion (i.e. $[Tl(I)] = [Tl^+]$); this assumption is also likely to be valid for the results of the independent studies presented in Table 3.

Regarding competing ions, the concentration of K^+ is particularly important to Tl^+ toxicity because of similarities in their ionic and

Table 3
Details and results of published studies on Tl(I) toxicity to fresh water primary producers, invertebrates and fish.

Tl added	Species	Medium	Measure; end point	Toxicity, μg l^{-1}	Reference
	Microalgae				
Not specified	*Chlamydomonas reinhardtii*	Buffered distilled water	O_2 evolution; IC_{50} (time unspecified)	3060	Overnell (1975)
Not specified	*Pseudokirchneriella subcapitata*	Not specified	Growth; 72 h IC_{25}	90	Pickard et al. (2001)
$Tl(I)NO_3$	*Pseudokirchneriella subcapitata*	M4	Growth; 72 h IC_{25}	160	Rickwood et al. (2015)
		M4 without K^+	Growth; 72 h IC_{25}	4.6	
Tl(I) standard	*Chlorella* sp.	Fraquil	Growth; 72 h EC_{50}	160	Hassler et al. (2007)
		Fraquil without K^+	Growth; 72 h EC_{50}	2.0	
	Macrophytes				
$Tl(I)_2SO_4$	*Elodea canadensis*	Buffered distilled water	Plant damage; 28 d IC_{50}	2000	Brown and Rattigan (1979)
	Lemna minor	Buffered distilled water	Plant damage; 28 d IC_{50}	8	
$Tl(I)NO_3$	*Lemna minor* L. clone St	Steinberg	Various growth parameters; 168 h EC_{50}	~300	Naumann et al. (2007)
$Tl(I)NO_3$	*Lemna minor* L. clone St	Steinberg	Frond abscission; 24 h $E_{FA}C_{50}$	440	Henke et al. (2011)
$Tl(I)CH_3OO$	*Lemna minor*	1/4 Steinberg	Growth/frond no.; 10 d EC_{50}	32 to 47	Kwan and Smith (1988)
	Invertebrates				
$Tl(I)NO_3$	*Hyalella azteca*	Dechlorinated tap water	Survival; 28 d LC_{25}	10	Borgmann et al. (1998)
		Dechlorinated tap water	Growth; 42 d EC_{25}	7.1	
		Artificial medium without K^+	Survival; 28 d LC_{25}	2.4	
		Artificial medium without K^+	Growth; 42 d EC_{25}	1.8	
$Tl(I)NO_3$	*Daphnia magna*	EPA "moderately" hard water	Survival; 48 h EC_{50}	1660	Lin et al. (2005)
	Ceriodaphnia dubia	EPA "moderately" hard water	Survival; 48 h EC_{50}	660	
Not specified	*Daphnia magna*	Not specified	Survival; 48 h LC_{50}	2010	Pickard et al. (2001)
	Ceriodaphnia dubia	Not specified	Survival; 168 h LC_{50}	370	
	Ceriodaphnia dubia	Not specified	Reproduction; 168 h IC_{25}	100	
$Tl(I)NO_3$	*Ceriodaphnia dubia*	M4	Neonate production; <144 h IC_{25}	160	Rickwood et al. (2015)
		M4 without K^+	Neonate production; <144 h IC_{25}	35	
	Fish				
$Tl(I)_2SO_4$	*Pimephales promelas* larvae	Well water	Survival; 96 h LC_{50}	860	LeBlanc and Dean (1984)
$Tl(I)NO_3$	*Pimephales promelas* larvae	Dechlorinated tap water	Survival; 168 LC_{50}	>500	Rickwood et al. (2015)
Not specified	*Oncorhynchus mykiss*	Not specified	Survival; 96 h LC_{50}	4270	Pickard et al. (2001)

consequent similarities in their biogeochemical characteristics and behaviours at the cellular level (Brismar, 1998). Thus, K^+ has been observed to reduce both the uptake and toxicity of Tl(I) in a range of aquatic organisms (Borgmann et al., 1998; Twiss et al., 2004; Hassler et al., 2007; Rickwood et al., 2015) and differences in its concentration among different media likely account for variations in Tl(I) toxicity observed for a given species (see results in Table 3). In the present study, evidence for the ameliorating role of K^+ on Tl toxicity is observed in the results of the *Daphnia* exposures conducted in OECD water and dechlorinated tap water. Here, Tl is more toxic to both species in tap water because its K concentration is about half of that in the synthetic medium.

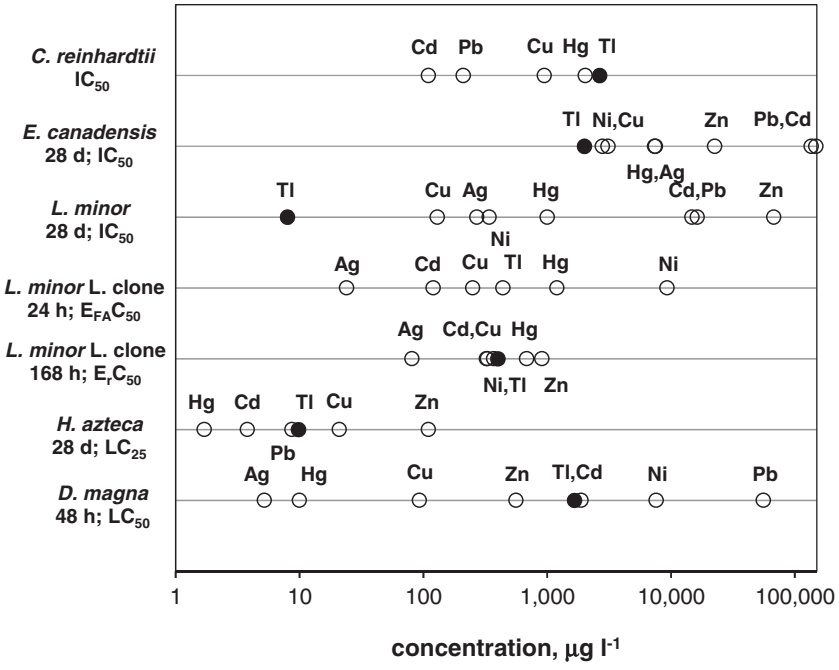

Fig. 7. A comparison of the acute toxicity of Tl(I) with the acute toxicities of other metals as derived from studies employing multiple metals (added individually) in specific tests (Overnell, 1975; Brown and Rattigan, 1979; Naumann et al., 2007; Henke et al., 2011; Borgmann et al., 1998; Lin et al., 2005).

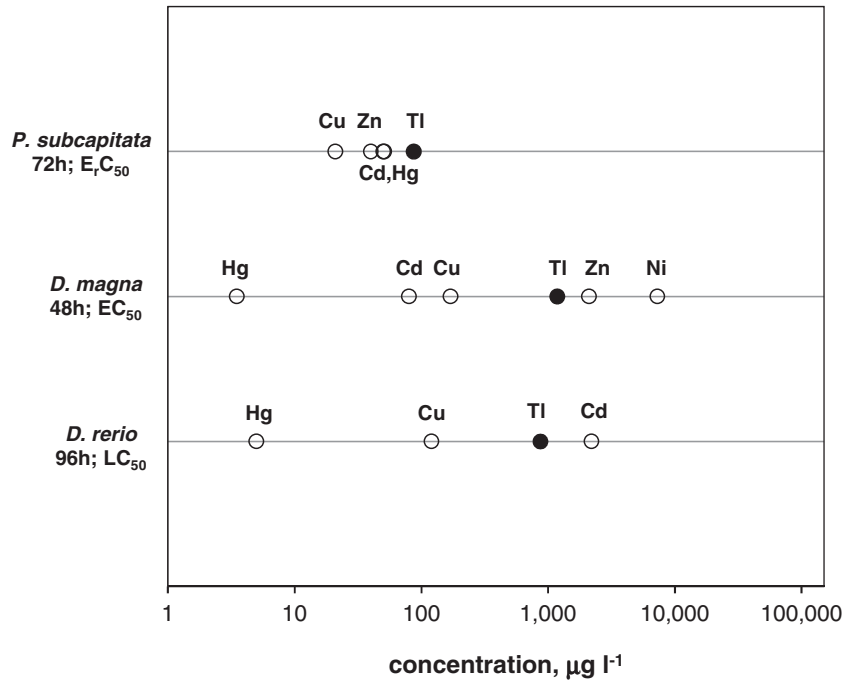

Fig. 8. A comparison of the acute toxicity of Tl(I), as derived in the present study, with the acute toxicities of other metals as established in independent studies employing the same tests and end-points (Martins et al., 2007; Rodrigues et al., 2013; Machado and Soares, 2014).

Given the effects of K^+, Tl toxicity may also be expressed in terms of its abundance relative to that of its analogue. On this basis, and using concentrations of K^+ in Table 1 and the data in Table 2, toxicity ranges from Tl^+ to K^+ ratios of 0.017 (72 h E_yC_{25} for *P. subcapitata*) to about 0.5 (48 h EC_{50} for *D. pulex* exposed in tap water). Regarding *D. magna*, when the results for tap water and OECD water in Table 2 are normalised to K^+, ratios for equivalent end-points are remarkably similar (EC_{50} ~ 0.36, EC_{25} ~ 0.26), suggesting that K^+ alone is responsible for differences in Tl toxicity between the two media. For *D. pulex*, results for the two media are closer on K^+ normalisation but ratios are greater (or Tl less toxic) in tap water (EC_{50} = 0.51, EC_{25} = 0.40) than in OECD water (EC_{50} = 0.33, EC_{25} = 0.25).

Based on toxicity and accumulation studies employing a microalga, cyanobacteria and rotifer, Hassler et al. (2007) suggest that below a threshold Tl^+ to K^+ ratio of 0.02 (or 0.004 on a molar basis), the aquatic toxicity of Tl to microorganisms will be suppressed. Our results are quantitatively consistent with this assertion for both invertebrates and early-life fish in that, in all cases, ratios eliciting a toxic response exceed 0.02. However, we note a Tl^+ to K^+ ratio for the NOEC to *P. subcapitata* of less than 0.01, and ratios derived from Borgmann et al. (1998) for the amphipod *H. azteca* and Brown and Rattigan (1979) for the macrophyte *L. minor* as low as 0.0036 and 0.002, respectively. Clearly, therefore, the threshold suggested by Hassler et al. (2007) does not apply to a wider range of aquatic organisms.

4.4. Regulatory context

The only environmental fresh water quality guideline for Tl that appears to have been developed with a clear rationale is that described by the Canadian Council of Ministers of the Environment (CCME, 1999). Here, a guideline of 0.8 µg l^{-1} was derived from the chronic (28 d, 50% whole plant damage) toxicity of Tl(I) to the macrophyte, *L. minor* (Brown and Rattigan, 1979), the most sensitive aquatic organism/end-point reported at the time, multiplied by a safety factor of 0.1. Since the publication of the guideline, more data have emerged on a wider variety of aquatic organisms, including toxicities lower than the concentration on which the guideline was based. Significantly, many subsequent studies have shown a clear dependence of Tl toxicity on the relative abundance of K^+, and toxic effects at concentrations of less than 2 µg l^{-1} in the absence of this cation in some cases. Thus, it would appear that the water quality guideline for Tl should be revised downward, and should factor in some dependence on K^+ concentration (or employ a Tl^+ to K^+ ratio; see Section 4.3).

Because we used three trophic levels with suitable end-points and timescales and undertook the assays according to established guidelines, the acute toxicity data generated in the present study satisfy accepted criteria for deriving a predicted no effect concentration (PNEC), or an environmental concentration at and below which adverse effects in exposed organisms are unlikely (Hahn et al., 2013). According to the European Union Water Framework Directive (European Communities, 2011), when only acute toxicity data are available for three trophic levels, a protective factor of 1000 should be applied to the lowest end-point concentration (as EC_{50} or LC_{50}) determined. Specifically, our 72 h E_rC_{50} for *P. subcapitata* yields a PNEC of 0.087 µg l^{-1}, or an order of magnitude lower than the CCME water quality guideline. Risk may then be evaluated by dividing a predicted (environmental) exposure concentration, PEC, by the PNEC, where a hazard quotient exceeding 1 indicates potential toxicity and it is advised that the chemical is further investigated on a site-specific basis (Hahn et al., 2013). Regarding rivers, a global average (background) aqueous concentration of Tl of about 0.007 µg l^{-1} has been estimated (Nielsen et al., 2005), resulting in a "global" hazard ratio of about 0.08. However, in areas impacted by historical or contemporary metal or coal mining, Tl concentrations in excess of 5 µg l^{-1} have been reported (Cheam, 2001; Casiot et al., 2011), resulting in a hazard ratio in excess of 50. On this basis, such regions would clearly be of concern regarding Tl contamination. However, as with the water quality guideline approach outlined above (CCME, 1999), this means of risk assessment does not account for water characteristics, and in particular the competitive effects of K^+.

5. Conclusions

This is the first study to determine the acute toxicity of Tl(I) to three trophic levels according to well-established OECD guidelines. The results reveal that the microalga, *P. subcapitata*, is more sensitive to Tl than either species of *Daphnia* tested or to the early life stages of

D. rerio. Our results suggest that a PNEC for Tl of about 0.09 μg l^{-1} is appropriate for freshwaters; this is an order of magnitude lower than the only available (Canadian) water quality guideline. However, given the ameliorating effects of K^+ on Tl^+ toxicity, it is recommended that further studies target the relationship between the two ions in this respect. Given the lack of measurements of Tl in the environment, but that concentrations are known to approach or exceed the PNEC in many regions affected by metal or coal mining, it is also recommended that Tl be included more widely in routine monitoring of water quality and that the metal be considered for inclusion into the European Union Water Framework Directive.

Conflict of interest

The authors declare that they have no conflicts of interest.

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