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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.