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Species sensitivity distribution approach to primary risk analysis of the metal pyrithione photodegradation product, 2,2′-dipyridyldisulfide in the Inland Sea and induction of notochord undulation in fish embryos

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A B S T R A C T

To carry out a primary risk assessment in the Inland Sea of Japan for 2,2′-dipyridyldisulfide [$(PS)_2$], a metal pyrithione photodegradation product, we used a methodology based on the species sensitivity distribution (SSD) estimated with a Bayesian statistical model. We first conducted growth inhibition tests with three marine phytoplankton species, *Tetraselmis tetrathele*, *Chaetoceros calcitrans*, and *Dunaliella tertiolecta*. We also performed acute and early life stage toxicity (ELS) tests with a teleost fish, the mummichog (*Fundulus heteroclitus*). The algal growth inhibition tests revealed that the 72-h EC_{50} ranged from 62 to 1100 μg/L. Acute toxicity tests with larval mummichogs revealed that the 96-h LC_{50} was approximately 500 μg/L based on the actual toxicant concentrations. ELS testing of $(PS)_2$ under continuous flow-through conditions for 50 days revealed that growth was the most sensitive endpoint, and both total length and body weight were significantly lower in the groups exposed to 27 μg/L $(PS)_2$ compared to the solvent control group. We determined a lowest observed effect concentration of 17 μg/L and a NOEC of 5.9 μg/L based on the actual toxicant concentrations. By using the ecotoxicity data (LC_{50} and EC_{50}) from this study and previous work, we calculated a hazardous concentration that should protect 95% and 99% of species (HC_5 and HC_1) based on the SSD derived with a Bayesian statistical model. The medians with 90% confidence intervals (parentheses) of the HC_5 and HC_1 were 31.0 (3.2, 101.8) μg/L and 10.1 (0.5, 44.2) μg/L, respectively. In the ELS test, about 80% of hatched larvae exposed to 243-μg/L $(PS)_2$ displayed a notochord undulation. To elucidate the cause of the notochord undulation, we carried out embryo toxicity tests by exposing embryos at various developmental stages to $(PS)_2$. Exposure to $(PS)_2$ through the entire gastrulae stage was important to induction of the morphological abnormality. Lysyl oxidase activity was significantly decreased in these embryos compared to the control group, a suggestion that lysyl oxidase-mediated collagen fiber organization, which is essential for notochord formation, is disrupted because of $(PS)_2$ toxicity. We also investigated the occurrence of $(PS)_2$ in water from several coastal sites of the Inland Sea and detected $(PS)_2$ at concentrations of <0.1–0.4 ng/L. Comparison of environmental concentrations to the HC values suggests that the current ecological risk posed by $(PS)_2$ in the Inland Sea is low. This is the first report of the detection of a metal pyrithione degradation product in the natural marine environment.

1. Introduction

Because of the restrictions on the use of organotin antifoulants in the 1980s, new antifouling biocides have been used on ship hulls. Metal pyrithiones (MePTs) such as copper pyrithione [CuPT; bis-(hydroxy-2(H)-pyridine thionate-O,S)-copper] and zinc pyrithione [bis-(hydroxy-2(H)-pyridine thionate-O,S)-zinc] are among the substitutes for organotin antifoulants frequently used as biocide components of the new antifouling paint products registered on the Japanese market (Okamura and Mieno, 2006). Reports have documented the toxicity of MePTs to several marine organisms, including a diatom, a polychaete tube warm, a crustacean, and an amphipod (Bao et al., 2008; Koutsaftis and Aoyama, 2006; Onduka et al., 2010). In addition, our previous studies revealed that the acute toxicity of CuPT to red sea bream, *Pagrus major*, a common inhabitant of coastal waters around Japan, is similar to that of tributyltin to some other marine fish species, such as juvenile Atlantic menhaden (*Brevoortia tyrannus*) and girella (*Girella*

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punctata) (Mochida et al., 2006), and CuPT reduced the growth of the mummichog *Fundulus heteroclitus* in a chronic toxicity study at less than 1 μg/L (Mochida et al., 2008). CuPT is thus markedly toxic to some marine fish species. However, insufficient consideration has been given to the ecological risk associated with MePT marine antifoulant degradation products, because MePTs are very light sensitive (Harino, 2004; Turley et al., 2000) and are thought to readily degrade through photolysis to nontoxic products in the natural environment (Turley et al., 2000). Maraldo and Dahllöf (2004), however, have pointed out that degradation of MePTs is negligible in seawater in the absence of exposure to sunlight. This finding suggests that MePTs are more persistent in turbid marine environments such as marinas and harbors, where the influence of light is restricted. Indeed, reports have already documented the detection of CuPT in sediment from fishing ports of Vietnam and Japan (Harino et al., 2006, 2007).

Sakkas et al. (2007) identified six MePT degradation products that were produced under simulated solar irradiation. Qualitative analysis indicated that 2,2′-dipyridyldisulfide [$(PS)_2$] would likely be one of the major photodegradation products in the natural environment (Sakkas et al., 2007). Although clarification of the environmental stability and fate of these products is incomplete, we detected $(PS)_2$ in seawater samples from a marina in the Inland Sea in our preliminary survey. In addition, Onduka et al. (2010) first reported the toxicity of MePT degradation products to several marine organisms, including a diatom, a crustacean, and a fish, and revealed that of six degradation products, $(PS)_2$ was the most toxic to red sea bream in acute toxicity tests. Collectively these results point to the urgency and importance of assessing the ecological risk of $(PS)_2$ to marine organisms in coastal areas of Japan.

The primary objective of the current study was to determine hazardous concentrations (HC) with the use of species sensitivity distribution (SSD) methodologies. SSD methodologies are widely used in probabilistic ecological risk assessment to determine the predicted no-effect concentration (PNEC) (Posthuma et al., 2002). The HC is the concentration of a toxic substance that is assumed to protect *p* percent of the species in a community. It is calculate by assuming a log logistic distribution of species sensitivities and developing an analytical expression for the concentration associated with the *p*th percentile of the distribution, which is called the HC for *p* percent of the species (Kooijman, 1987; Van Straalen and Denneman, 1989). For decision-making in ecological risk assessment, a cutoff value of 5% for *p*, which means 5% of the species are left unprotected, is often used. Although objection was frequently raised regarding to the value, a variety of ecotoxicity data supported well for a numerical similarity between HC_5 and environmental concentrations at which the first adverse effects start to become apparent (Posthuma et al., 2002).

To accumulate toxicity data for the SSD, we first carried out algae-growth inhibition tests with three marine phytoplankton species, *Tetraselmis tetrathele*, *Chaetoceros calcitrans*, and *Dunaliella tertiolecta*. We also carried out early life stage toxicity (ELS) tests and acute toxicity tests with a marine teleost fish, the mummichog. Because the early life stages of fish (from embryonic development to larvae) are more sensitive to chemical toxicity than the adult stage, the ELS test is a reliable predictor of chronic toxicity values obtained from full life-cycle toxicity testing (McKim, 1977; Van Leeuwen et al., 1985). Van Straalen and Denneman (1989) used no-observed effect concentrations (NOEC) to calculate SSDs, because they considered NOECs to be more representative for field situations. However, the use of NOECs as endpoints for the SSD methodology is still under debate, and the concept of SSD can be implemented equally well with LC_{50} values, NOECs, and EC_{10} values (Posthuma et al., 2002). Because only limited NOEC data for $(PS)_2$ were available, we used LC_{50} and EC_{50} values obtained from previous studies (Mochida et al., 2011; Onduka et al., 2010) and this study to estimate the SSD of $(PS)_2$.

The analysis of a single normal SSD fit and judgments of goodness-of-fit can be problematic, when only a limited number of ecotoxicity data are available. Under such conditions it is important to assess the uncertainty of the SSD and its derived quantities (Posthuma et al., 2002). Aldenberg and Jaworska (2000) analyzed the uncertainty of a normal SSD with Bayesian statistical inference and showed numerically confidence limits of the mean values. The advantages of Bayesian statistics, including their ability to treat uncertainty in an explicit and consistent way and to update inferences with new data (Ellison, 1996), recommends their use as a standard tool in the environmental sciences. Applications of the Bayesian approach include the derivation of SSDs (Grist et al., 2006; Hayashi and Kashiwagi, 2010, 2011; Hickey et al., 2008) and estimation of hazardous concentrations (Fox, 2010). In the present study, we estimated the HC_5 and also HC_1 of an SSD (i.e., 5th and 1st percentile of the SSD) derived with Bayesian inference, and also compared the HC values to NOECs from the algae-growth inhibition tests and the ELS test to judge suitability of HC values as candidates of PNEC.

During the ELS tests we found that one morphological abnormality, distortion of the trunk derived from a notochord undulation, occurred in hatched mummichog larvae, an indication that the abnormality was already present during embryonic development. To investigate the critical stage for the induction of the abnormality, we exposed embryos at various stages of development to $(PS)_2$ and examined the occurrence of abnormal embryos. A recent study revealed that treatment with another MePT degradation product, 2-mercaptopyridine-*N*-oxide (HPT), inhibited lysyl oxidase (LO) activity, the result being notochord undulations in zebrafish embryos (*Danio rerio*) (Anderson et al., 2007). LO inhibition-mediated induction of notochord undulation has also been reported in zebrafish embryos exposed to the insecticide cartap [*S,S*′-(2-dimethylaminotrimethylene)bis(thiocarbamate)] (Zhou et al., 2009). Therefore, we also investigated the possible involvement of LO activity inhibition in the notochord abnormality.

In addition, we investigated the occurrence of $(PS)_2$ in water from several coastal areas of the Inland Sea, especially in the vicinity of ship yards, harbors, and marinas, and we carried out a primary ecological risk assessment of $(PS)_2$ of the natural marine environment based on a comparison of environmental concentrations to the HC values.

2. Materials and methods

2.1. Animals

T. tetrathele, *C. calcitrans*, and *D. tertiolecta* were kindly provided by the Tohoku National Fisheries Research Institute (Miyagi, Japan). Algae were cultured in a 1-L glass bottle with *f*/2 medium (Guillard and Ryther, 1962) under static-renewal conditions on a 14:10 light–dark cycle in a temperature-controlled (20 ± 1 °C) growth chamber (MLR-350; Sanyo, Osaka, Japan). The light source consisted of three ultraviolet-screened fluorescent tubes (FL40S N-EDL NU; Matsushita Electric Industrial, Osaka, Japan) that provided a photon flux of 39 ± 8 $\mu mol\ m^{-2}\ s^{-1}$ in the wavelength range 360–670 nm. The *f*/2 growth medium was prepared from natural seawater filtered through sand, activated carbon, and a glass fiber filter (GF/C, Whatman, Maidstone, UK; hereafter "filtered seawater").

The Arasaki strain of the mummichog (Shimizu, 1997) has been bred in our laboratory for several years. Ten adult mummichogs, both male and female with secondary sexual characteristics and weighing approximately 10 g, were kept for at least 7 months