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Derivation of predicted no-effect concentration and ecological risk for atrazine better based on reproductive fitness

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ABSTRACT

Atrazine (ATZ) is an herbicide most commonly used in China and other regions of the world. It is reported toxic to aquatic organisms, and frequently occurs at relatively high concentrations. Currently, ATZ has been proved to affect reproduction of aquatic species at much lower levels. So it is controversial to perform ecological risk assessment using predicted no-effect concentrations (PENCs) derived from traditional endpoints, which fail to provide adequate protection to aquatic organisms. In this study, PNECs of ATZ were derived based on six endpoints of survival, growth, behavior, biochemistry, genetics and reproduction. The PNEC derived from reproductive lesion was 0.044 μg ATZ L^{-1}, which was obviously lower than that derived from other endpoints. In addition, a tiered ecological risk assessment was conducted in the Taizi River based on six PNECs derived from six categories of toxicity endpoints. Results of these two methods of ecological risk assessment were consistent with each other, and the risk level of ATZ to aquatic organisms reached highest as taking reproductive fitness into account. The joint probability indicated that severe ecological risk rooting in reproduction might exist 93.9% and 99.9% of surface water in the Taizi River, while 5% threshold (HC_5) and 1% threshold (HC_1) were set up to protect aquatic organisms, respectively. We hope the present work could provide valuable information to manage and control ATZ pollution.

1. Introduction

Atrazine (ATZ) is probably the most widely applied herbicide all over the world, and one of the most frequent pollutants in surface and ground waters. It is used diffusely to kill certain weeds, primarily in corn but also in sugarcane, sorghum, orchard and landscape vegetation to a certain extent. In the past several decades, China has been the major producer and user of ATZ. Approximately, 2000 t of ATZ are produced at least per year in China. So far ATZ is still in use particularly in northeast and north China and other regions (Zheng et al., 2014). Due to its persistence and mobility in the environment, ATZ occurs at μg/L concentrations in water environments (Ryberg et al., 2010; Shelley et al., 2012) and has been detected in most surface waters, reservoirs and lakes of China at considerably high concentrations (Gfrerer et al., 2002; Ren et al., 2002; Ta et al., 2005; Wang et al., 2002; Yan et al., 2005; Zheng et al., 2014).

Actually, exposure to ATZ may lead to a hazardous effect to ecosystem and human health. Besides, in the past few years, it has been the research hotspot whether ATZ has endocrine disrupting properties at environmentally relevant low concentrations. Previous researches have suggested that ATZ had hormonal effects both in vitro and in vivo for different species including *Daphnia magna*, *Biomphalaria alexandrina*, *Rana pipiens* and *Pimephales promelas* (Bringolf et al., 2004; Gammon et al., 2005; Gustafson et al., 2015; Hayes et al., 2003, 2002; Moore and Lower, 2001; Palma et al., 2009; Spanò et al., 2004; Tillitt et al., 2010). Other studies, however, have found no endocrine disrupting effects on invertebrates (*Hyalella azteca*), amphibians (*Xenopus laevis*) and mammals (Male rats) (Jablonowski et al., 2011; Jooste et al., 2005; Ralston-Hooper et al., 2009; Solomon et al., 2008; Spolyarich et al., 2010; Trentacoste et al., 2001). It seemed that this problem was not resolved completely at that time. However, with the in-depth study of the endocrine disruption of ATZ and the joint efforts of all parties in the last few years, the Office of Environmental Health Hazard Assessment (OEHHA) officially confirmed ATZ had endocrine

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disruptor activity especially in reproduction toxicity, and added ATZ to the Proposition 65 list on July 15, 2016 (OEHHA, 2016). In general, ATZ could induce production of vitellogenin (VTG) which is a precursor protein of egg yolk in plasma and hemolymph and is often used as a biomarker of environmental estrogens, decrease fertility and fecundity to aquatic organisms, change concentrations of steroids in blood, and induce other sexual development problems such as feminizing of males or masculinizing effects on females (Bringolf et al., 2004; Freitas and Rocha, 2012; Papoulias et al., 2014). Due to the potential toxicity of ATZ, deriving predicted no-effect concentration (PNEC) and conducting ecological risk of ATZ is necessary to protect the aquatic species.

An important step to assess ecological risk of contaminants is to determine the PNEC, a maximum concentration under which the ecosystem cannot be influenced. Generally, PNEC is derived based on laboratory toxicity experiments (particularly for chronic) following well-defined protocols or guidelines (Jin et al., 2011). Despite a mass of toxicity data of ATZ available on various organisms such as Daphnia, fish, insect and algae, the final decision was not made for ATZ about PNEC derivation before July 2016 since the mode of action of ATZ to aquatic species was still debating (Shelley et al., 2012). Water quality criteria (similar to PENC) for ATZ has been derived by United States Environmental Protection Agency (USEPA, 2001, 2003), Canadian Council of Ministers of the Environment (CCME, 1999) and Ministry of Environmental Protection of the People's Republic of China (MEP, 2002). However, many of them derived a toxicity threshold with an application factor. As for CCME, the water quality guideline value of ATZ for the protection of freshwater life is 1.8 μg/L. It was derived by multiplying the lowest maximum acceptable toxicant concentration (MATC) of 17.9 μg/L by this application factor of 0.1. In 2001, USEPA has also developed a criterion continuous concentration (CCC) of 12 μg/L applying acute to chronic ratio (that is the application factor) to protect aquatic lives (USEPA, 2001). In the revised version in 2003, USEPA corrected the CCC considering the ecosystem effects of ATZ to aquatic plants. These derived water quality criteria did not take the reproduction into account. Meanwhile, the latest guidelines of USEPA recommended that the derivation of the freshwater chronic criterion (equal to PNEC) should be reexamined if ATZ was conclusively demonstrated a significant reproductive effect to aquatic species (has endocrine disruption effects) (USEPA, 2003). Also Jin et al. (2013) and Caldwell et al. (2008) indicated that traditional PENC was incapable of providing adequate protection for aquatic species since some pollutants have been proved to affect reproductive fitness at relatively low concentrations rather than survival and growth. Therefore, it is urgent to derive the PENC and conduct ecological risk for ATZ based on reproduction toxicity.

In this study, PNECs of ATZ were derived using a statistical method based on different toxicity endpoints such as mortality, growth, biochemistry, behavior, genetics and reproduction. Additionally, a tiered ecological risk assessment approach of both deterministic method and probabilistic method based on reproductive toxicity was carried out in the Taizi River. The present research would provide a reference for decision makers to revise the ATZ standard and assess ATZ risk in aquatic ecosystem.

2. Materials and methods

2.1. Environmental concentrations

2.1.1. Study area

The Taizi River is a vital water resource to surrounding areas located in Liaoning Province in Northeast China (122°25' – 124°55′E and 40°28' – 41°39'N) (Fig. 1). It originates from Hongshilazi mountain and finally flows into the Bohai Sea with a total length of 413 km (Liu et al., 2011). As a typical tributary of the Liao River, it lies within a temperate continental monsoon climate zone, with annual average temperature ranging from 5 °C to 9 °C. Annual average evaporation ranges from 1100 to 1600 mm in the basin, which is greater than annual average rainfall of approximate 804 mm (Wan et al., 2012). The Taizi River basin is an important agricultural zone in northeast China with the main crops including corn, wheat, rice, sorghum and so on. However, with the rapid development and urbanization, a large number of pollutants containing trace organic pollutants have been released into the Taizi River. Ecological system in the basin has been deteriorated (Bu et al., 2014).

2.1.2. Sample collection and analysis

A of 19 surface water samples (S1-S19) were collected from the Taizi River and its branches in June 2013 (Fig. 1). Global positioning system (GPS) was applied to record the locations of sampling sites. Surface water was taken from 1 m below the water surface with precleaned stainless metal bucket, and filtrated through 0.70 μm glass filter membranes. The filtered water was stored in 4-L glass amber. Samples were transported to the laboratory on ice and stored in the refrigerator, in a dark environment at 4 °C.

2.1.3. Chemicals analysis

ATZ were extracted through a solid-phase extraction (SPE) system with the modified method presented by a previous research (Dean et al., 1996; Gfrerer et al., 2002; Zheng et al., 2014; Zhou et al., 2006) The Supelclean LC-18 cartridges were sequentially preconditioned by methanol (5 mL) and ultrapure water (5 mL), and ATZ in surface water were loaded onto cartridges using SPE. After the cartridges were dried by the vacuum (1 h), 6 mL of 1:1 dichloromethane/n-hexanes and 6 mL of 1:1 dichloromethane/acetone were used to elute the cartridges. The extract was concentrated to 0.7 mL, and anhydrous sodium sulfate was added to remove residual water. Then the extract was spiked with 200 ng phenanthrene-d_{10} as an internal standard and concentrated to 1.0 mL using hexane. Finally, a gas chromatograph-mass spectrometer (Agilent GC-6890A, Agilent MS-5975C) was used to analyze the concentration of ATZ in a selected ion mode.

2.1.4. Quality assurance and Quality Control (QA/QC)

All chemical analyses and data processing were strictly conducted following the quality assurance and quality control procedures (Gao et al., 2008; Zheng et al., 2014). ATZ was quantitatively determined using retention time and peak area with references to a calibrated standard curve. Seven different concentrations of working standards were used to construct the calibration curve, and a good linearity was observed ($r^2 \geq 0.99$). For every batch of ten samples, a solvent blank and a procedural blank were run in sequence to check interferences and circumvent cross-contaminations. Method detection limit (MDL) of ATZ, which was 7.32 ng/L, was set as three times of the noise from the baseline. Average recoveries of ATZ in water samples were in the range of 85–114%.

2.2. Effect assessment

2.2.1. Toxicity data collection and selection

Toxicity data of ATZ were collected and selected from the ECOTOX database (http://cfpub.epa.gov/ecotox/), China Knowledge Resource Integrated Database (http://www.cnki.net/), PubMed and Web of Science databases. The key words included atrazine, risk assessment, water quality criteria, criterion maximum concentration, criterion continuous concentration, predicted no effect concentration, toxicity, different toxicity endpoints, environmental exposure, detection, concentration and the Taizi River. The government documents, research reports and other available data were also captured from literature retrieval.

As for acute toxicity data of ATZ, median effect concentration (EC_{50}) was used as the measurement endpoint. If the EC_{50} was not able to be obtained, the median lethal concentration (LC_{50}) was used instead. In terms of chronic toxicity data of ATZ, no observed effect concentration