Chemosphere 209 (2018) 857–865

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Changes in thyroid status of *Menidia beryllina* exposed to the antifouling booster irgarol: Impacts of temperature and salinity

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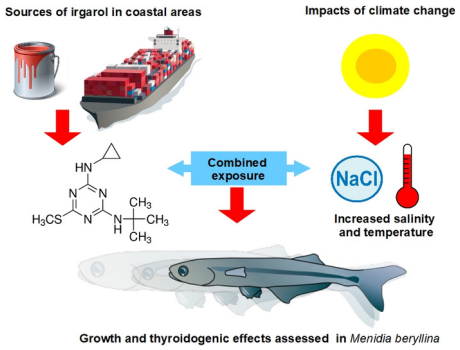
HIGHLIGHTS

- Thyroid effects of irgarol assessed in *Menidia beryllina*.
- Condition factor of *M. beryllina* affected by irgarol.
- Thyroid targets of irgarol affected by temperature and salinity.
- Potential Adverse Outcome Pathway for irgarol is provided.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 March 2018
Received in revised form
1 June 2018
Accepted 24 June 2018
Available online 26 June 2018

Handling Editor: Jim Lazorchak

Keywords:
Biocides
Climate change
Estuary
Endocrine disrupting chemicals
Fish endocrinology
Inland silversides

ABSTRACT

The triazine-based herbicide irgarol is widely used in antifouling systems as an algicide and has been detected recently in multiple coastal environments. Studies evaluating sub-lethal responses of fish following exposure to irgarol are limited. Moreover, impacts of climate change on fish endocrinology may also contribute to the sublethal toxicity of irgarol. We assessed the effects of irgarol on thyroid endpoints in juveniles of *Menidia beryllina* under two different treatments of salinity (10 and 20 ‰) and two temperatures (10 and 20°C). Condition factor coefficients (K) of animals were significantly affected by 0.1 to 10 μg/L of irgarol at the higher temperature. Levels of T3 were changed in whole body homogenates from both temperatures at 10‰ following exposure to 1 to 10 μg/L. T4 levels were altered only at 10°C when animals were treated with 1 to 10 μg/L (10 ‰), and in 0.1 and 10 μg/L (20 ‰). Increased transcripts of deiodinase enzymes at 10 °C may be impacted by salinity and alter thyroid hormone homeostasis. Impact on gene expression of thyroid (α and β) and growth hormone receptors were also determined. Our results highlight the relevance of environmental variable that may impact the ecological risk of irgarol in estuarine systems.

1. Introduction

Antifouling compounds are used in coating systems to prevent biocorrosion and unwanted development of biofouling on man-

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https://doi.org/10.1016/j.chemosphere.2018.06.152
0045-6535/

made surfaces including boats, vessels, off-shore formation and other constructions in aquatic environments (Chambers et al., 2006; Thomas and Brooks, 2010). Due to regulatory restrictions of organotin paints (OT), the use of OT-free antifouling systems has increased as an alternative to reduce the toxicity of antifouling systems to non-target organisms (Hall et al., 2009). Such paints contain biologically active compounds known as "organic boosters', which includes different biocides that are incorporated into the coating formulation formulations (Mochida and Fujii, 2009).

Since the mid-1980s the triazine-based herbicide irgarol (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) has been widely used in antifouling systems as an algaecide (Hall et al., 2009; Mochida and Fujii, 2009). Since usage has increased, detection of irgarol has been observed in coastal environments (Bao et al., 2013). Irgarol is the most common detected antifouling booster worldwide, with environmental concentrations ranging from <1 ng/L to 1 μg/L (Konstantinou and Albanis, 2004). In Southern California for example, levels of irgarol in water were measured up to 254 ng/L in sites nearby recreational marinas (Sapozhnikova et al., 2013). Irgarol has a reported half-life of 100–350 days (7 ± 3 days for estuarine waters), with moderate water solubility (7 mg/L) and lipophilicity (log K_{ow} = 3.95) (Thomas et al., 2002; Konstantinou and Albanis, 2004; Sapozhnikova et al., 2009, 2013).

The mode of action of irgarol is similar to other triazine herbicides, in that it blocks electron transport during photosynthesis by inhibiting the energy transfer of photosystem II (PSII), reducing both CO_2 uptake and carbohydrate production, which causes the starvation of the plant (Ebert et al., 1976). The toxicity of irgarol in estuarine and marine organisms is well documented in the literature and most studies have focused on acute and chronic effects endpoints in bioassays for algae, invertebrates and fish (Konstantinou and Albanis, 2004; Bao et al., 2011; Castro et al., 2011; Perina et al., 2011; Mai et al., 2013). However, studies on sub lethal effects are limited with one study showing immunosuppression in the ascidian *Botryllus schlosseri* (Cima and Ballarin, 2012) and changes in the fatty acid composition of Asian sea-bass *Lates calcarifer* (Ali et al., 2015). Regarding endocrine responses, the anti-estrogenic activity of irgarol was reported using a yeast-based in vitro assay (Westlund and Yargeau, 2017). Recent studies demonstrate that the antifouling booster diuron, which also acts in PSII, affect the thyroid system, growth, and development of aquatic organisms, including tadpoles American bullfrog *Lithobates catesbeianus* and the estuarine fish *Menidia beryllina* (Freitas et al., 2016; Moreira et al., 2018).

Silversides from the species *M. beryllina*, are the most common fish originated from West Atlantic inhabiting coastal environments from marshes to estuaries, and was introduced to Pacific coastal ecosystems (Middaugh and Hemmer, 1992). The species is a secondary consumer feeding on zooplankton and represents an important link in the marine food web as a prey item of other fish from higher links (Gleason and Bengtson, 1996). *M. beryllina* is also a eurythermic (tolerance range from 2.9 to 30 °C) and euryhaline (tolerance range from 0 to 35‰) (USEPA, 2002), and recently it has been selected as a model for estuarine fish in the assessment of endocrine disrupting chemicals (EDCs) in coastal zones (Brander et al., 2016; Cole et al., 2016).

Shifts in environmental conditions including those induced by climate change events can also affect the toxicity of contaminants (Schiedek et al., 2007; Keller et al., 2015). In California's San Francisco Estuary-Watershed (SFEW) for example, climate change projections indicate extreme conditions of summers and winters (Cloern et al., 2011; Wagner et al., 2011). Such models project the decrease of freshwater input combined with seawater intrusion, as a result of sea level rising, increasing thus water temperature and salinity in the estuarine system. Considering this scenario, we assessed the effects of irgarol on thyroid function of estuarine fish using *M. beryllina* as a model under two conditions of temperature and salinity. Thyroid hormone (TH) endpoints were evaluated in juveniles and genes involved in TH pathways such as deiodinases enzymes (Dio1 and Dio3), growth (GHR) and thyroid hormone receptors (TRα and TRβ) and were evaluated in fish's body homogenates following treatments. Molecular responses were combined to condition factor to determine if an Adverse Outcome Pathway could be formulated for irgarol.

2. Materials and methods

2.1. Animals and experimental design

We used sexually immature juveniles with 50 days of age, with 11.42 ± 1.27 mm length and 0.016 ± 0.006 g of weight. Animals were obtained from commercial cultures (Aquatic BioSystems Inc., Fort Collins, CO) and kept for 10 days of acclimation in experimental conditions of control preceding the experiments:10‰ of water salinity, 10 °C of temperature and photoperiod of 12:12 h light:dark. Fish were fed brine shrimp nauplii once per day at 5:00 p.m. (USEPA, 2002). After the 10-day acclimation period, a semi-static exposure was performed at 10 °C to evaluate the thyroidogenic effects of irgarol in *M. beryllina*. We tested two factors in each exposure: (I) salinity: 10‰ and 20‰, in order to assess salinity changes in oligohaline waters and (ii) irgarol: solvent control and three concentrations (0.1, 1 and 10 μg/L). Such concentrations were selected based on levels detected in samples from marinas of Southern California and other areas of East Coast from Florida to North Carolina (Hall et al., 2005; Sapozhnikova et al., 2013). Three months later, a new batch of fish with 50 days of age was purchased and a second exposure was performed at 20 °C with the same salinities and levels of irgarol, based on variations in estuarine conditions tolerated by estuarine fish and temperature changes predicted by SFEW models (Cloern et al., 2011).

Analytical grade irgarol (Sigma-Aldrich) was purchased for preparing stock solutions in ultra-pure water and using 0.01% MeOH as a solvent. Irgarol exposure systems were assembled in 4L tanks settled in a water bath (100 L capacity) with control of temperature performed *via* water-cooled chillers connected to thermostats. Sea salt aliquots were dissolved in dechlorinated water to achieve the selected salinity and then irgarol at nominal concentrations was added in a final volume of 3L. Two tanks *per* treatment were set up and kept in a cycle of 12–12 h (light-dark) with constant aeration. Following the 10 days of acclimation, 10 fish *per* tank were assigned to the respective treatment and exposed to irgarol for 15 days. Fish were fed on brine shrimp nauplii once *per* day at 5:00 p.m. at least 2h preceding the water changes (90% of total volume), which were performed every 48 h. Water pH (7.5 ± 0.5) and NH3 (below 0.01 mg/L) were monitored before water changes. No changes in feeding rate or mortality were observed during the experimental exposures and after that, fish were euthanized using 300 mg/L of tricaine methanesulfonate (MS-222). Measurements of total length and wet weight were recorded and animals were frozen on liquid nitrogen to be stored at -80°C until the analysis. These procedures followed the Animal Use Protocol (AUP), approved by UC Riverside Institutional Animal Care and Use Committee (IACUC).