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Environmental concentrations of irgarol, diuron and S-metolachlor induce deleterious effects on gametes and embryos of the Pacific oyster, *Crassostrea gigas*

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ABSTRACT

Irgarol and diuron are the most representative "organic booster biocides" that replace organotin compounds in antifouling paints, and metolachlor is one of the most extensively used chloroacetamide herbicides in agriculture. The toxicity of S-metolachlor, irgarol and diuron was evaluated in Pacific oyster (*Crassostrea gigas*) gametes or embryos exposed to concentrations of pesticides ranging from 0.1× to 1000×, with 1× corresponding to environmental concentrations of the three studied pesticides in Arcachon Bay (France). Exposures were performed on (1) spermatozoa alone (2) oocytes alone and (3) both spermatozoa and oocytes, and adverse effects on fertilization success and offspring development were recorded. The results showed that the fertilizing capacity of spermatozoa was significantly affected after gamete exposure to pesticide concentrations as low as 1× of irgarol and diuron and 10× of metolachlor. The offspring obtained from pesticide-exposed spermatozoa displayed a dose-dependent increase in developmental abnormalities. In contrast, treating oocytes with pesticide concentrations up to 10× did not alter fertilization rate and offspring quality. However, a significant decline in fertilization success and increase in abnormal D-larvae prevalence were observed at higher concentrations 10× (0.1 μg L^{-1}) for S-metolachlor and 100× for irgarol (1.0 μg L^{-1}) and diuron (4.0 μg L^{-1}). Irgarol, diuron and S-metolachlor also induced a dose-dependent increase in abnormal D-larvae prevalence when freshly fertilized embryos were treated with pesticide concentrations as low as concentration of 1× (0.01 μg L^{-1} for irgarol or S-metolachlor, and 0.04 μg L^{-1} for diuron). The two bioassays on *C. gigas* spermatozoa and embryos displayed similar sensitivities to the studied pesticides while oocytes were less sensitive. Diuron, irgarol and S-metolachlor induced spermiotoxicity and embryotoxicity at environmentally relevant concentrations and therefore might be a threat to oyster recruitment in coastal areas facing chronic inputs of pesticides.

1. Introduction

Pesticides are commonly used throughout the world as agents for controlling adverse species in agriculture or as antifouling biocides in paint formulations (Banks et al., 2005; Okamura et al., 2000a). They may enter the environment by a variety of routes including through urban (parking lots and residential areas) and agricultural (treated farming areas) run-off, leaching or spray drift, contaminated soils and aquatic sediments. Pesticide contamination has now been widely recorded in waters and sediments from various European estuaries, coastal areas, and lakes (Sargent et al., 2000; Thomas et al., 2000). Once pesticide residues have been discharged in aquatic ecosystems, they can persist from a few months to several years (Hayes and Mlaws, 1991; Ranke, 2002).

Aquatic organisms accumulate pollutants from contaminated water or by ingesting contaminated food; therefore the pollutants may lead to the contamination not only of the aquatic organisms themselves but also of the entire ecosystem, including mankind, through the food chain. There are also numerous compounds such as pesticides that do not bioaccumulate at all and thus do not leave clear fingerprint, but do injure living organisms. For this reason bioindicators, such as invertebrate species, are very useful to study the effects of pollutants in the aquatic environment (His et al., 1999). Bioassays are also very useful to measure the toxicity of chemicals or effluents and to estimate the safe concentration of pollutants which is acceptable in the environment. The early developmental stages of

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bivalves are particularly suitable for these kinds of test because they are easily produced and maintained in controlled laboratory conditions and because they develop quickly in a few hours or days, depending on the species (Beiras and His, 1994; Chapman and Morgan, 1983). They are also sensitive to a large range of pollutants (His et al., 1999). For all these reasons they are widely used to provide accurate biological criteria on the toxicity of pollutants, in particular pesticides (Armstrong and Millemann, 1974).

According to Apte and Day (1998), pesticides used in grass and broadleaf weed control in agriculture or in antifouling boat paint are the most ecotoxicologically relevant to marine species. S-metolachlor is one of the most intensively used chloroacetamide herbicide in agriculture and is moderately toxic to freshwater and estuarine animals (Lizotte et al., 2009; Cook and Moore, 2008; Jin-Clark et al., 2008; Wan et al., 2006). For instance, the low acute toxicity of metolachlor exposure was found for *Oncorhynchus* sp. and *Daphnia magna*, with 24 h-LC_{50} values of 19,000–44,000 μg L^{-1} and 80,000 μg L^{-1}, respectively. Metolachlor is regularly detected in European surface waters during the crop-growing season (Buser et al., 2000). Diuron is a substituted urea herbicide and has been associated with general weed control on land. The use of this compound as an herbicide has been restricted in France since 2002 (JO n°80 April 5, 2002). Diuron has also been used as an antifouling agent in many European countries (Boxall et al., 2000; Martinez et al., 2001). This compound is commonly detected in areas of high boating or yachting activity and is considered to be relatively persistent in seawater (Konstantinou and Albanis, 2004), with a slow breakdown (from one month to one year) (Okamura, 2002) and half-lives of about 60 days (Harino et al., 2005). In Dutch and British coastal areas, diuron is present at higher concentrations than irgarol and other biocides (Lamoree et al., 2002; Thomas et al., 2002). Depending on the time or concentration of exposure and the considered species, diuron has shown toxic effects on various organisms such as algae, crustaceans and fish (Fernández-Alba et al., 2001, 2002; Okamura, 2002). Irgarol is a herbicidal additive used in copper-based antifouling paints and it is estimated that its overall residence time in the marine systems is over 10 years (Ranke, 2002). If so, irgarol can be considered as a persistent organic pollutant. High levels of irgarol were reported in April–May when the high boating season starts at the west coast of Sweden (Dahl and Blanck, 1996). Early studies reported that although irgarol was found to be highly toxic to algae and higher plants, as it is a potent inhibitor of photosynthesis (Dahl and Blanck, 1996; Macinnis-Ng and Ralph, 2003; Nyström et al., 2002; Owen et al., 2002), it is comparatively less toxic to marine bacteria and crustaceans as well as in fish cell culture (Okamura et al., 2000b, 2002). However, to our knowledge, the literature relating to the toxic effects of metolachlor, irgarol and diuron on marine invertebrate species, particularly the Pacific oyster, is still limited.

The Arcachon Bay is a marine lagoon of the Atlantic Ocean on the Southwest coast of France in the Aquitaine region. The bay covers an area of about 150 km^2 at high tide and 40 km^2 at low tide and receives mainly freshwater inputs from the Leyre River (about 20 m^3 s^{-1}). This area is a well-known place for oyster farming with about 8000 to 10,000 tons produced each year and provides 60% of the French production of juvenile oysters (Auby and Maurer, 2004). For several years (2007–2011), low larval recruitment, reduced spat fall and increased oyster mortality events were observed in the Arcachon Bay suggesting impaired reproduction, mortality or developmental defects at early life stages (Maurer et al., 2011). Exposure to pollutants could partly account for the reduction of early life stages of the Pacific oyster. Indeed, early life stages of oysters including gametes, embryos, and larvae are known to be particularly sensitive to contaminants such as metals, pesticides, PAHs and persistent organic contaminants (Akcha et al., 2012; His et al., 1999).

Table 1
Pesticides concentrations (ng L^{-1}) determined for reference seawater from the Arcachon Bay and working solutions used in this study.

	Measured		
	Diuron	Irgarol	Metolachlor
Arcachon Bay[a]	5–40	2.5–22	2.5–5
Reference seawater[b]	1	2	1
Working solution 10,000×	406,000	75,000	90,000

[a] Auby and Maurer (2004).
[b] Seawater collected in Eyrac (Arcachon Bay) in April 2011.

The most recent data available for the Arcachon Bay (Table 1) indicate concentrations up to 40 ng L^{-1} for diuron, 22 ng L^{-1} for irgarol and 5 ng L^{-1} for metolachlor (Auby and Maurer, 2004).

Pacific oyster embryos have been used for more than one decade to assess both chemical toxicity and water quality (His et al., 1999; Beiras and His, 1994; Geffard et al., 2001). It is well-known that Pacific oyster (*C. gigas*) gametes are freely spawned and that fertilization occurs externally in the seawater. Therefore, exposure to pollutants can occur as soon as gametes are released in the water column. In this study, chemical exposure was performed either on gametes or on freshly fertilized embryos from *C. gigas*. The purpose of this study was both (i) to evaluate the toxicity of commonly used herbicides on *C. gigas* and (ii) to compare the sensitivity of both gamete types and embryos to pesticide exposure.

2. Materials and methods

2.1. Chemicals and reference seawater

Analytical grade S-metolachlor, irgarol and diuron were purchased from Sigma–Aldrich (St Quentin Fallavier, France). Seawater was collected from Eyrac station in Arcachon Bay (SW of France), an area where oysters reproduce naturally. Immediately after sampling, seawater was sieved (0.2 μm mesh) to eliminate debris and microorganisms. The filtered seawater (FSW) was stored at 4 °C and was used within 3 days. A few hours before the experiment, FSW was filtered again at 0.2 μm.

2.2. Animals

Mature oysters (*C. gigas* Thunberg, 1793) came from a commercial hatchery specialized in the year-round production of mature oysters (Guernsey Sea Farms, UK). Oysters were kept at around 10 °C for transportation and then acclimatized in FSW for 2 h at 18 °C before the beginning of the experiments. All oysters were used within 3 days.

2.3. Pesticide solutions

Since irgarol and diuron have a relatively low water solubility (7 mg L^{-1} and 42 mg L^{-1} respectively), their stock solutions (100 mg L^{-1}) were prepared in DMSO, whereas the stock solution of metolachlor (100 mg L^{-1}) was prepared in Milli-Q water. 100 μg L^{-1} (10,000×) working solutions were obtained from the dilution of the stock solutions in FSW. Five test solutions at 0.1×, 1×, 10×, 100× and 1000× were then obtained by diluting the working solutions in FSW. 1× represents the environmental concentration of pesticides in Arcachon Bay according to Auby and Maurer, 2004 (see Table 1). The negative control consisted of FSW with 0.01% DMSO (the highest DMSO concentration used in the test solutions) except for metolachlor exposure where control was carried out at a final Milli-Q water concentration of 0.01%. All treatments were performed using four replicates in 24 well-plates.