Available online at www.sciencedirect.com
SCIENCE DIRECT®
Ecotoxicology and Environmental Safety 61 (2005) 221–229
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Rapid communication

# Oxidative stress and genotoxic responses to resin acids in Mediterranean mussels

C. Gravato*, M. Oliveira, M.A. Santos

*Department of Biology, Animal Physiology/Ecotoxicology Sector, University of Aveiro, 3810-193 Aveiro, Portugal*

Received 29 December 2003; received in revised form 2 November 2004; accepted 27 December 2004
Available online 8 February 2005

**Abstract**

This study represents the first attempt to investigate the genotoxic effects and oxidative stress of resin acids in Mediterranean mussels (*Mytilus galloprovincialis* Lmk.). Mussels were exposed to 2.7 μM abietic acid (AA) and dehydroabietic acid (DHAA) for 6, 12, 18, and 24 h. Gill and hepatopancreas conjugation activity, antioxidant defense system, lipid peroxidation (LPO), and DNA damage were determined as reduced glutathione (GSH), glutathione *S*-transferase (GST) activity, glutathione peroxidase (GPx) activity, catalase (CAT) activity, LPO, and DNA strand breaks. AA caused significant GST inhibition in mussel gills at 12, 18, and 24 h. Activity of the antioxidant enzymes, namely, GPx and CAT, was inhibited at 24 and 18 h, respectively, in mussel gills. A significant increase in gill LPO was observed at 24 h. The DNA integrity of mussel hepatopancreas significantly decreased after 12 and 24 h exposure to AA. A significant increase in LPO was observed after 6 h exposure to DHAA, in either mussel gills or hepatopancreas. DNA integrity was significantly decreased in mussel hepatopancreas after 12 and 24 h exposure to DHAA. AA induced oxidative damage and genotoxicity in mussels, because it promoted increases in LPO in gills and DNA strand breaks in hepatopancreas. DHAA promoted oxidative damage and genotoxicity in mussels, as significant increases were observed in LPO in gills and hepatopancreas and in DNA strand breaks in hepatopancreas.


*Keywords:* Abietic acid; Dehydroabietic acid; Resin acids; Enzymatic and nonenzymatic antioxidants; Lipid peroxidation; DNA strand breaks; *Mytilus galloprovincialis*

## 1. Introduction

Large amounts of bleached kraft pulp and paper mill effluents (BKPPMEs) are discharged into the aquatic environment causing serious ecological and toxicological effects in the receiving ecosystems. A complex mixture of urban and industrial effluents, including those resulting from BKPPMEs, is presently discharged through a submarine pipe outlet into the Aveiro (Portugal) coastal waters, contaminating its water and sediments (Gravato and Santos, 2002a, b, 2003).

Pulp and paper mill effluents contain more than 300 compounds that affect water quality, as short- or long-term exposure to BKPPMEs induces a wide range of histopathological and physiological changes in fish (Andersson et al., 1988; Bengtsson et al., 1988; Härdig et al., 1988; Lindström-Seppä and Oikari, 1989; Munkittrick et al., 1991; Santos and Pacheco, 1995; Adams et al., 1992; Pacheco and Santos, 1999).

A significant percentage of pulp and paper mill effluent toxicity in fish is attributable to resin acids (RAs) and their transformation products (Nikinmaa and Oikari, 1982; Oikari and Lindström-Seppä, 1990; Pacheco and Santos, 1997a, b, 1999; Gravato and Santos, 2002a, b). RAs, such as abietic acid (AA) and dehydroabietic acid (DHAA), are commonly found at concentrations of 40–2500 μg/L in treated effluents and 4–14 μg/L in receiving waters (Holmbom and Lehtinen, 1980; Oikari and Kunnamo-Ojala, 1987; Kaplin et al.,

*Corresponding author.
*E-mail address:* gravatoc@bio.ua.pt (C. Gravato).

0147-6513/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2004.12.017

1997; Leppänen et al., 1998). Several investigations have been performed on RA toxicity in fish, revealing its liver biotransformation (Oikari et al., 1983; Rabergh et al., 1992; Pacheco and Santos, 1997a, 1999; Gravato and Santos, 2002a, b), and genotoxicity-inducing potential (Das and Nanda, 1986; Pacheco and Santos, 1997a, b, 1999; Gravato and Santos, 2002a, b; Maria et al., 2004a, b). However, a relationship between liver biotransformation induction by RAs and subsequent adverse effects, namely, their genotoxicity, was not consistently established, suggesting that a bioactivation process, such as redox cycling, may also be involved. Stimulated reactive oxygen species (ROS) production and resulting oxidative damage might be a toxicity mechanism in aquatic organisms exposed to pollution (Di Giulio et al., 1989; Livingstone et al., 1990, 1993; Winston and Di Giulio, 1991; Lemaire and Livingstone, 1993; Ahmad et al., 2000; Santos et al., 2004). The extent to which oxyradical generation produces biological damage is dependent on the effectiveness of antioxidant defenses (Michiels and Remacle, 1988). A number of free radical scavengers, such as glutathione, together with antioxidant enzymes, such as catalase (CAT, EC. 1.11.1.6) and glutathione peroxidase (GPx, EC. 1.11.1.9), play a crucial role in maintaining cell homeostasis. These antioxidant responses, among others, have been proposed as biomarkers of contaminant-mediated oxidative stress in a variety of marine organisms, including mussels (Livingstone et al., 1992; Gamble et al., 1995; Regoli et al., 1998).

The effects of pulp mill effluents or their constituents, such as RAs, on mussels and other bivalve mollusks are almost unknown. Wu and Levings (1980) observed abnormal growth rate and reproductive activities in mussels transplanted near a pulp mill effluent. A recent histopathological and enzyme cytochemical study showed that rosin, which contains the prototype RAs found at varying concentrations in pulp mill effluents (Liss et al., 1997), is toxic to *Mytilus edulis* (Fahraeus-Van Ree and Payne, 1999). However, the aforementioned research works were the only attempts to evaluate the effects of pulp mill constituents on mussels. Thus, the use of mussels as monitoring species around pulp mills and determination of the toxic effects of RAs are of great importance (Salazar, 1996; Fahraeus-Van Ree and Payne, 1999).

This study was performed to improve knowledge of the responses of marine mussels to pulp mill effluent compounds, namely, RAs. With this perspective, the present research work was carried out to investigate and compare *Mytilus galloprovincialis* responses in gills and hepatopancreas after 6, 12, 18, and 24 h exposure to 2.7 μM AA. This particular concentration was used because of its genotoxic effects after short-term exposure of fish (Gravato and Santos, 2002a, b). Therefore, this research represents the first attempt to investigate the genotoxic effects and oxidative stress of RAs in mussels, in a short-time experiment under controlled laboratory conditions. The following parameters were measured: (1) activity of glutathione *S*-transferase (GST) as a phase II conjugation enzyme; (2) reduced glutathione (GSH) as a conjugation substrate and free radical scavenger; (3) activities of CAT and GPx as antioxidant enzymes; (4) lipid peroxidation (LPO) as an oxidative damage effect; (5) and DNA strand breaks as a genotoxic effect.

## 2. Material and methods

### 2.1. Chemicals

Abietic acid (AA) was purchased from Sigma Chemical Company (USA). Dehydroabietic acid (DHAA) was obtained from Helix Biotech Corporation (Canada). All other chemicals used were of analytical grade and obtained from Sigma Chemical Compnay (USA), Boehringer Mannheim GmbH (Germany) and E. Merck-Darmstadt (Germany). The salt mixture used to prepare the artificial seawater was obtained from Sera Meersaltz, Heinsberg (Germany).

### 2.2. Test animals

Mediterranean mussels (*M. galloprovincialis* Lmk.) specimens were caught in Vagueira Beach (Aveiro, Portugal). Mussels with $3.5 \pm 0.5$ cm (mean $\pm$ SD) in shell length were transported to the laboratory and allowed to recover for 2 weeks in 15-L glass tanks. During recovery, mussels were kept at 17 °C (room temperature) in aerated and continuously filtered saltwater (35 g/L) without being fed.

### 2.3. Experimental protocols

Mussels were exposed to nothing (control) and 2.7 μM AA or DHAA for 6, 12, 18, and 24 h, without being fed. The experiments were carried out at 17 °C in aerated and continuously filtered saltwater (35 g/L) under a natural photoperiod. Five mussels per experimental condition were dissected, and hepatopancreas and gills were divided in two halves. Each half of both tissues was individually frozen in liquid nitrogen, stored at −80 °C until either further homogenization or DNA isolation. Samples from each mussel tissue were individually analyzed ($n = 5$).

### 2.4. Biochemical analysis

One-half of each tissue was removed from refrigeration and homogenized (1:15) in 0.1 M phosphate buffer