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Anguilla anguilla L. genotoxic and liver biotransformation responses to abietic acid exposure

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Abstract

Adult eels (*Anguilla anguilla* L.) were exposed for 8, 16, 24, and 72 h to 0, 0.1, 0.3, 0.9, and 2.7 μM abietic acid (AA). Genotoxicity was measured as erythrocytic nuclear abnormalities (ENA), as well as DNA strand breaks in blood and liver. Liver cytochrome P450 (P450) content, liver ethoxyresorufin *O*-deethylase (EROD), and glutathione *S*-transferase (GST) activities were determined as biotransformation biomarkers. Liver alanine transaminase (ALT) activity was also measured as an indication of tissue damage. Low AA concentrations, such as 0.1 and 0.3 μM, result in a delayed induction of *A. anguilla* L. liver EROD activity, whereas the higher AA concentration (2.7 μM AA) also has a delayed effect probably as a consequence of liver tissue high inhibitory concentration. The current eel liver GST activity results demonstrate that only low AA concentrations promote liver increases in GST, whereas high AA concentrations, such as 0.9 and 2.7 μM, do not alter it. The results concerning eel liver ALT activity indicate that significant liver damage is induced by high AA concentrations, such as 2.7 and 0.9 μM. The eel ENA result analysis reveals that AA is a weak ENA inducer in *A. anguilla* L. Blood DNA integrity results suggest that low AA concentrations promote late decreases in blood DNA integrity; nevertheless, high AA concentrations are early blood genotoxic inducers compared with low AA doses. According to the present research results with respect to eel liver DNA damage, all of the AA exposure concentrations decreased liver DNA integrity.

Keywords: Genotoxicity; DNA strand breaks; Liver ethoxyresorufin-*O*-deethylase; Erthrocytic nuclear abnormalities; Eel

1. Introduction

Pulp and paper mill effluents (PMEs) include a variety of compounds that are highly toxic to aquatic organisms (Walden and Howard, 1977). Soft wood is resinous, containing resin acids (RAs) in its tissue, part of which is released to the effluent during pulping and bleaching processes (Morales et al., 1992; Kaplin et al., 1997; Leppänen et al., 1998).

A reasonable number of studies concern the toxic effects of PME constituents, namely, RAs. These diterpene acids are known to be relevant contributors to the toxicity of PMEs and strong toxicants to fish (Nikinmaa and Oikari, 1982; Oikari et al., 1983). Abietic acid (AA) and dehydroabietic acid (DHAA) are among the most abundant RAs in those effluents (Oikari et al., 1980).

Biochemical, physiological, and structural effects on fish exposed to PMEs as a whole or to their compounds have been widely documented (Owens, 1991). Toxic sublethal effects include developmental damage, growth disturbance, altered weight of organs, liver biotransformation enzyme induction or inhibition, liver and blood DNA breakage, carbohydrate and protein metabolism changes, osmoregulation, and hematologic alteration (Andersson et al., 1987; Lindström-Seppä and Oikari, 1989, 1990a, b; Bengtsson, 1991; Owens, 1991; Söderg-ren, et al., 1992; Räbergh et al., 1992, Maria et al., 2003a).

Gravato and Santos (2002a) demonstrated that AA inhibites liver ethoxyresorufin *O*-deethylase (EROD) activity, in juvenile sea bass (*Dicentrarchus labrax*), after 2 h exposure to 0.05 μM. According to Pacheco and Santos (1997) a significant total EROD activity induction was observed in glass eels (*Anguilla anguilla* L.) after 3 days exposure to 0.3 μM AA. Previous research studies by Pacheco and Santos (1999) demonstrated liver EROD induction in adult eels after 3 days exposure to 0.1, 0.3, and 0.9 μM AA.

Anguilla anguilla L. has been found to be sensitive to polynuclear aromatic hydrocarbon (PAH) (Pacheco and Santos, 2001b; Maria et al., 2002), PAH-like compound

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(Teles et al., 2002; Maria et al., 2003b), PMEs (Pacheco and Santos, 1999, Maria et al., 2003a), RAs (Pacheco and Santos, 1997, 1999), and contaminated harbor water (Pacheco and Santos, 2001a; Maria et al., 2003c) in genotoxicity screening.

The ENA assay was revealed to be a sensitive and easy mutagenic/clastogenic test, efficient in either laboratory or field studies on PMEs and their main components, such as AA and DHAA (Pacheco and Santos, 1999). Induction of erythrocytic micronuclei (EMNs) and eryhthrocytic nuclear abnormalities (ENAs) in juvenile sea bass (*Dicentrarchus labrax*) was also demonstrated in laboratory experiments after 2, 4, 6, and 8 h exposure to AA (Gravato and Santos, 2002a, b). Despite the previous 3-day genotoxicity study carried out in fish species exposed to AA, the relation between induction of genotoxicity as DNA strand breaks, ENAs, and biotransformation in adult *A. anguilla* L. has not been consistently established for short exposures and needs to be studied.

From this perspective, the present research work intends to study genotoxic and biotransformation effects of AA (0, 0.1, 0.3, 0.9, and 2.7 μM) on *A. anguilla* L. over during 8, 16, 24, and 72 h. The genotoxic responses were measured as blood and liver DNA strand breaks and ENAs. Liver biotransformation (phases I and II) induction was also measured as EROD and glutathione *S*-transferase (GST) activities. Hematological parameters such as hemoglobin concentration (Hb), red blood cell number (RBC), and the ratio of Hb concentration to RBC (Hb/RBC) were also determined.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade, obtained from Sigma Chemical Company (USA), Boheringer–Mannheim GmbH (Germany), and E. Merck–Darmstadt (Germany). Abietic acid (AA) was obtained from Sigma Chemical Company (USA).

2.2. Fish

Anguilla anguilla L. (eel) with an average weight of 50 g (silver eel) were collected from the Aveiro Lagoon, Murtosa (Portugal). The eels were transported in anoxia and acclimated to laboratory conditions in aerated, filtered, and dechlorinated tap water in 200-L aquaria for 1 week, at 20°C.

2.3. Experimental protocols

The experiment took place in aquaria with 80 L of clean and dechlorinated tap water. The eels were not fed during the experimental protocol. Eels were exposed for 8, 16, 24, and 72 h to AA concentrations of 0 (control), 0.1, 0.3, 0.9, and 2.7 μM Each experiment was carried out using test groups of five eels ($n = 5$). The AA was previously dissolved in 1 mL of dimethyl sulfoxide (DMSO) and added to the water in the experimental aquaria. AA solution was not replaced during the whole exposure period. Fish were killed by decapitation; their blood and liver were sampled. Whole blood was used for red blood cell (RBC) count and hemoglobin (Hb) determination, whereas blood smears were prepared for ENA assay. Each liver was divided into two halves; one was immediately frozen in liquid nitrogen and stored at −20°C for later determination of liver EROD, P450, GST, and ALT, while the other half was placed in TNES–urea (8 M) buffer with proteinase K solution (the final concentration was 0.8 mg/mL) for the DNA isolation procedure (Genomic DNA Purification Kit, Fermentas). Fresh blood samples, after centrifugation and plasma separation, were stored overnight at 4°C in TNES–urea (8 M) buffer for DNA isolation. Blood and liver DNA extraction was carried out for the DNA strand break assay.

2.4. Genotoxicity tests

Genotoxicity was tested using the frequency of ENAs and the DNA alkaline unwinding assay. The ENA test was carried out in mature eel erythrocytes, according to the procedures of Schmid (1976), Carrasco et al. (1990), Smith (1990) as adapted by Pacheco and Santos (1996), and Ayllón and Garcia-Vazquez (2001). Each final result in the ENA test is presented as the mean value (‰) of the sums for all the individual lesions including *notched* nucleus, which corresponds to an appreciable depth into the nucleus that does not contain any nuclear material, observed and scored in 1000 cells per fish blood smear.

Blood and liver DNA integrity measurements (%) were performed according to Rao et al. (1996), with minor modifications. Data from the blood and liver DNA unwinding assay are expressed as F values (%) determined by applying the following equation: [(double stranded DNA)/(double stranded DNA + single-stranded DNA)] × 100.

2.5. Biochemical analysis

2.5.1. Liver ethoxyresorufin O-deethylase

Microsomes were obtained according to the methods of Lange et al. (1993) and Monod and Vindimian (1991) as adapted by Pacheco and Santos (1998). Liver EROD activity was measured as described by Burke and Mayer (1974). Results are expressed as picomoles per minute per milligram of microsomal protein.

2.5.2. Liver cytochrome P450 content

P450 content was quantified by measuring the absorbance spectra in the range 490–450 nm as described by Hermens et al. (1990).

2.5.3. Liver glutathione S-transferase (GST)

GST activity was determined as described in Habig et al. (1974) and Lemaire et al. (1996), with 1-chloro-2,4-dinitro benzene (CDNB) as substrate. The assay was prepared in the cuvette and was carried out in a 2-mL mixture of 0.2 M phosphate buffer (pH 7.4), 0.2 mM CDNB, and 0.2 mM reduced glutathione (GSH). The reaction was initiated by sample addition. The increase in absorbance at 340 nm was recorded at 25°C for 3 min.

2.5.4. Liver alanine transaminase assay

ALT activity was measured according to Reitman and Frankel (1957) in the supernatant resulting from liver microsomal isolation (Santos et al., 1990; Pacheco and Santos, 1999). Results are expressed as units per gram of protein.

2.5.5. Protein measurement

Microsomal protein content and supernatant protein concentration were determined according to the biuret method (Gornall et al., 1949), using bovine serum albumin (E. Merck–Darmstadt, Germany) as standard.

2.6. Hematology

Blood hemoglobin concentration (g/100 mL) was determined spectrophotometrically according to the cyan–methemoglobin method (van Kampen and Zijlstra, 1961) and the number of red blood cells (RBC) per cubic millimeter of blood was counted with a Neubaeur chamber (cell counter) after dilution (1:300) of the blood sample with an isotonic salt solution.

2.7. Statistical analysis

The results were log transformed and expressed as means ± SE. Statistica software (SigmaStat 2.03) was used for statistical analyses. All data were first tested for normality and homogeneity of variance to meet statistical demands. Variance analysis was used to compare results between fish groups, followed by Tukey test (Zar, 1996). Differences between means were considered significant at $P<0.05$.

3. Results

3.1. Liver phase I and II biotransformation responses

Anguilla anguilla L. liver EROD activity significantly ($P<0.05$) increased at 24 and 72 h exposure to 0.1 and 0.3 AA μM, when compared with controls (Fig. 1). The highest AA concentration (2.7 μM) promoted a significant ($P<0.05$) increase in liver EROD activity at 24 h exposure. Significant ($P<0.05$) increases in liver EROD activity were also observed at 8, 16, 24, and 72 h exposure to 0.9 μM AA compared with controls. Maximum values of liver EROD activity were observed at 72 (2.26-fold), 24 (2.69-fold), 16 (7.06-fold) and 24 (3.38-fold) exposure to 0.1, 0.3, 0.9, and 2.7 μM AA, respectively (Fig. 1). Eel liver P450 content was not significantly altered during the experiment (Fig. 2). A significant ($P<0.05$) increase in liver GST activity was observed at 24 h to 0.1 μM AA, as well as at 72 h exposure to 0.1 and 0.3 μM AA (Fig. 3).

3.2. Liver damage

Anguilla anguilla L. liver ALT activity significantly ($P<0.05$) decreased at 24 and 72 h exposure to 0.9 μM AA, as well as at 8, 16, 24, and 72 h to 2.7 μM AA (Fig. 4).

3.3. Hematological parameters

A significant ($P<0.05$) decrease in Hb was observed at 72 h to 0.1, 0.9, and 2.7 μM AA, whereas a significant ($P<0.05$) increase in Hb was observed at 8 h exposure to 2.7 μM AA, as well as at 16 h to 0.3 and 0.9 μM AA (Fig. 5). The ratio of Hb to RBC significantly ($P<0.05$) increased at 16 and 24 h exposure to 0.3 and 0.9 μM AA, respectively (Fig. 6). However, a significant ($P<0.05$) decrease in Hb/RBC was observed at 72 h exposure to 2.7 μM AA.

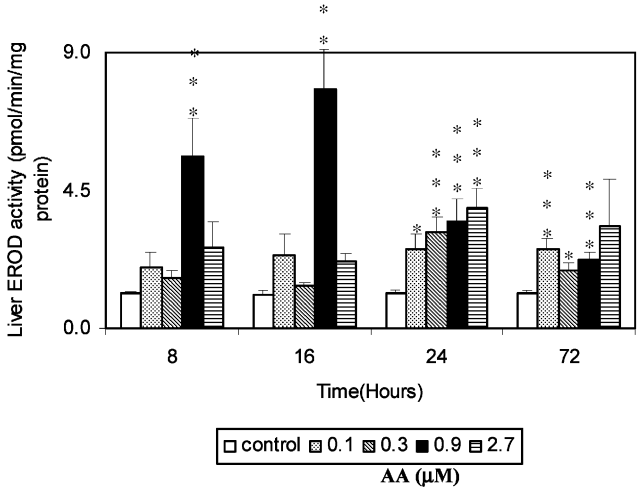

Fig. 1. A. *anguilla* L. liver EROD activity induction [log (pmol/min/mg protein)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

3.4. Genotoxicity responses

Anguilla anguilla L. ENA frequency significantly increased at 24 and 72 h exposure to 2.7 μM AA, compared with controls (Fig. 7). Moreover, the maximum ENA value was observed at 72 h (4.8-fold) exposure to 2.7 μM AA. A significant ($P<0.05$) decrease in blood DNA integrity was observed at 24 h to 0.1 and 0.3 μM AA, as well as at 72 h to 0.1 μM AA (Fig. 8), whereas after 8 h exposure we also observed a significant ($P<0.05$) blood DNA integrity decrease into 0.9 and 2.7 μM AA, as well as a significant ($P<0.05$) decrease in blood DNA at 16 h exposure to the highest AA concentration (2.7 μM AA). Concerning liver DNA integrity, a significant ($P<0.05$) decrease was observed at 24 and 72 h exposure to 0.1 μM AA (Fig. 9). Eel liver DNA integrity was significantly ($P<0.05$) decreased at 8

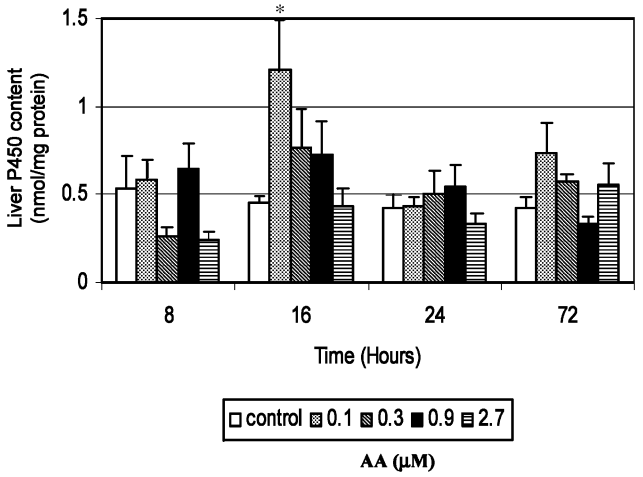

Fig. 2. A. *anguilla* L. liver cytochrome P450 content [log (nmol/mg protein)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

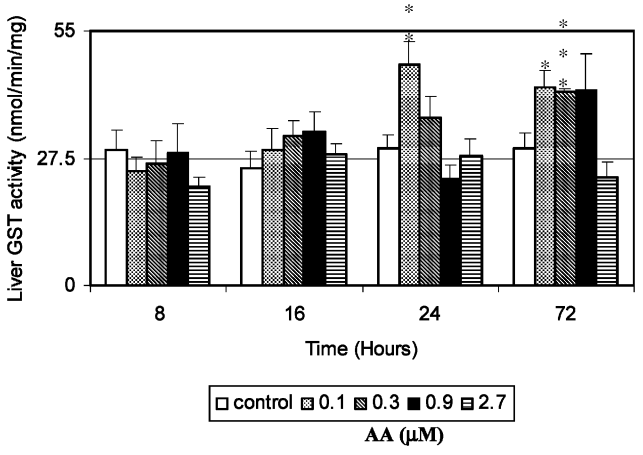

Fig. 3. A. *anguilla* L. liver glutathione *S*-transferase (GST) [log (nmol/min/mg protein)] activity after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

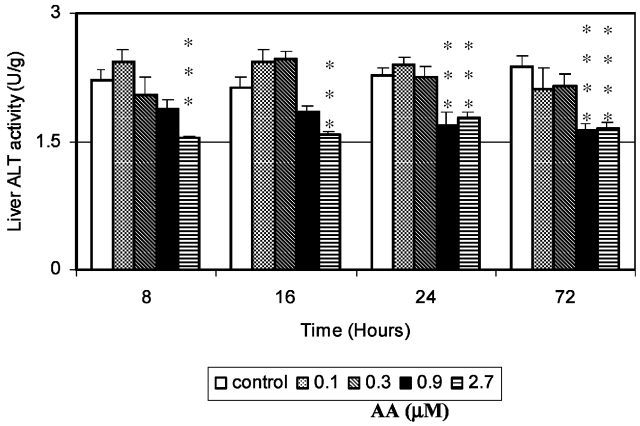

Fig. 4. A. *anguilla* L. liver alanine transaminase (ALT) [log (U/g)] activity after 8, 16, 24, and 72 h exposure to AA-0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

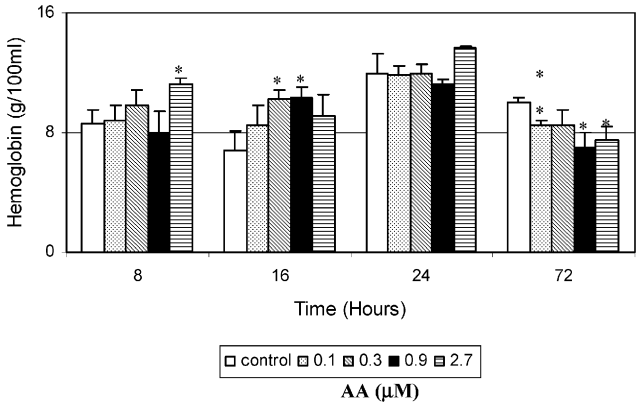

Fig. 5. A. *anguilla* L. hemoglobin (Hb) concentration [log (g/100 mL)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

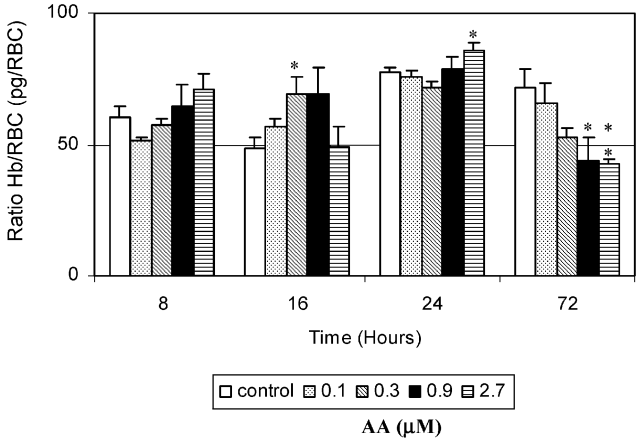

Fig. 6. A. *anguilla* L. ratio of Hb to RBC [log (pg/RBC)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

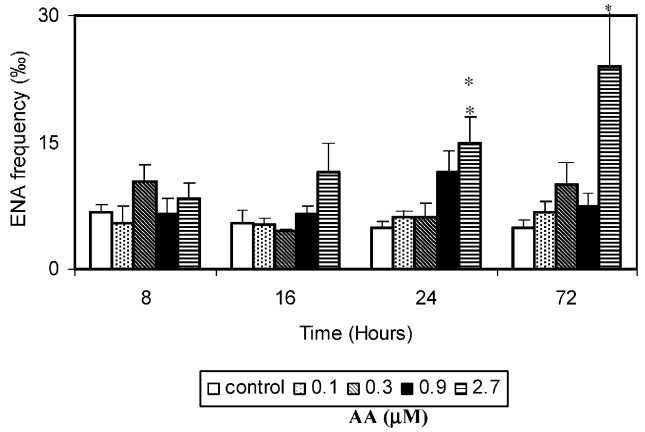

Fig. 7. A. *anguilla* L. ENA frequency [log (‰)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

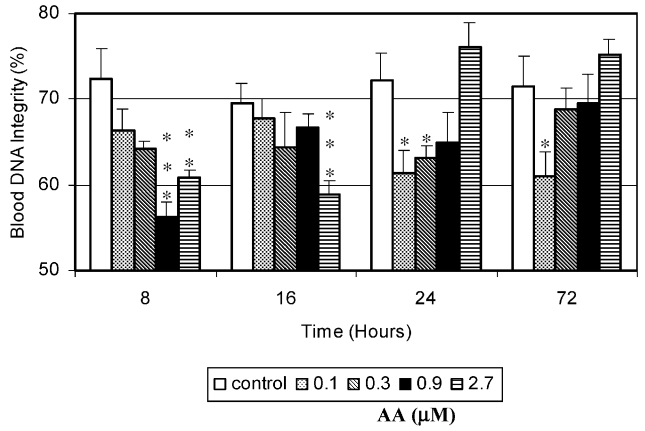

Fig. 8. A. *anguilla* L. blood DNA integrity [log (%)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

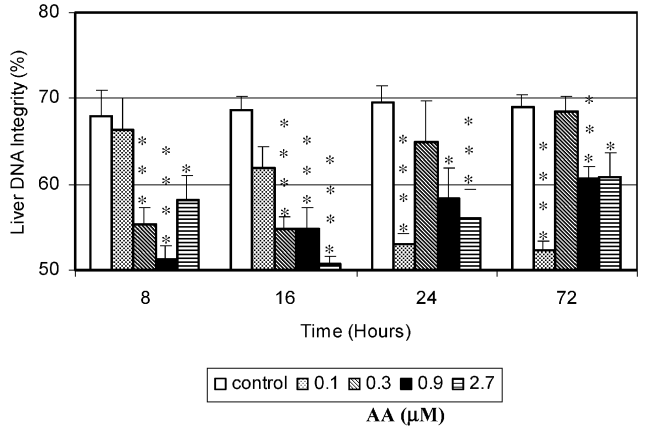

Fig. 9. A. *anguilla* L. liver DNA integrity [log (%)] after 8, 16, 24, and 72 h exposure to AA 0, 0.1, 0.3, 0.9, and 2.7 μM. Values represent means ± SE. Differences from control: $^{*}P<0.05$.

and 16 h exposure to 0.3, 0.9, and 2.7 μM AA, whereas, a significant ($P<0.05$) decrease in liver DNA integrity was also observed at 24 and 72 h exposure to 0.9 and 2.7 μM AA (Fig. 9).

4. Discussion

According to Pacheco and Santos (1999) a significant increase in liver EROD increase is observed in adult eels (*Anguilla anguilla* L.) after 3 days of treatment with one 14.7 μmol/kg intraperitoneal injection of AA, and exposure to 0.1, 0.3, and 0.9 μM water-diluted AA. However, Gravato and Santos (2002a, b) demonstrated that short-term exposures (2 and 6 h) to low AA saltwater concentrations (0.0125 and 0.05 μM) inhibit juvenile sea bass liver EROD activity.

The present research study demonstrated a delayed and slight induction of liver EROD activity in *A. anguilla* L. at 24 h exposure to 0.1 and 0.3 μM AA compared with 0.9 μM AA at 8 h, suggesting that AA exposure concentrations lower than 0.9 μM AA have a delayed effect. However, the low, delayed increase in *A. anguilla* L. liver EROD activity at 24 h exposure to 2.7 μM AA, compared with 0.9 μM AA, is probably due to the inhibitory effect of early high AA concentration. Comparison of eel and sea bass liver EROD activities, and also with the present results, suggests that different fish species and age spans may be associated with different sensitivities to AA exposure.

Elevated liver EROD activity values were also demonstrated in *A. anguilla* L. exposed from 8 to 72 h to 0.9 μM benzo[*a*]pyrene (BaP), a model polycyclic aromatic compound, acting as a strong liver EROD inducer (Maria et al., 2002). The same authors also showed that 2.7 μM BaP exerts a lower induction of liver biotransformation (Phase I) than 0.9 μM BaP in eels, suggesting an inhibitory effect at high BaP concentrations (Maria et al., 2002). Teles et al. (2002) also demonstrated that 0.9 β-naphthoflavone (BNF), a potent P450 mixed-function oxygenase (MFO), promotes elevated liver EROD activity compared with 2.7 μM BNF.

Previous research work with bleached kraft pulp and paper mill effluents (BKPPME) demonstrated that liver EROD activity increases in eels at 8 and 16 h exposure to 25% BKPPME, as well as at 16, 24, and 72 h exposure to 50% BKPPME (Maria et al., 2003a), suggesting that AA is among the most abundant RAs in BKPPME (Oikari et al., 1980; Subtil et al., 1984).

Liver GST activity has been reported as a biomarker for the environmental impact assessment of organic xenobiotics that generate oxidative stress (Rodriguez-Ariza et al., 1991; Livingstone, 1998). The current eel liver GST activity results demonstrated that only low

AA concentrations promote increases in liver GST, whereas high AA concentrations, such as 0.9 and 2.7 μM, do not alter it. An increase in liver GST was previously demonstrated in *A. anguilla* L after 72 h exposure to 50% BKPPME by Maria et al. (2003a). As GST is involved in xenobiotic detoxification and excretion of xenobiotics and their metabolites, its increased activity in liver may indicate development of a defensive mechanism to cope with pulp mill effluents and reactive organic pollutants. Nevertheless, Soimasuo et al. (1995) demonstrated that in caged whitefish (*Coregonus lavaretus* L.) exposed to bleached kraft mill effluent (BKME), liver GST and UDP-GT activity did not increase. However, a gradual and distance-related decrease in conjugated chlorophenolics (CPs) in bile was detected in that fish species under the previous exposure conditions. Other researchers have demonstrated that BKME components such as CPs and RAs may accumulate to high levels in bile, other fluids, and certain fish tissues (Oikari and Kunnamo-Ojala, 1987; Kierkegaard and Renberg, 1988; Lindström-Seppä and Oikari, 1989; Söderström and Wachtmeister, 1992).

Our experimental results concerning eel liver ALT activity indicate that significant liver damage is induced by high AA concentrations, such as 0.9 and 2.7 μM. This last event may also support the absence of increased liver GST activity and decreased liver EROD activity at 72 h compared with 24 h exposure to the same concentrations. Pacheco and Santos (1999) also observed decreased liver ALT activity in adult *A. anguilla* L. after 3 days exposure to 2.7 μM AA.

Among the identified pulp and paper mill effluents contaminants, a significant number of chemicals are classified as clastogens, mutagens, and carcinogens (Houk, 1992). In vitro short-term tests (Ames test and SOS chromotest) have demonstrated that mainly chlorinated, low-molecular-weight chemicals are responsible for genotoxicity (Nylund et al., 1994). Despite Pacheco and Santos' (1999) demonstration of the genotoxic potential of AA in adult eels as reflected in increased ENA frequency at 72 h exposure to 0.3, 0.9, and 2.7 μM in adult eels, the present experimental results partially agree with those studies, since we observed an increase in ENA frequency only at 24 and 72 h exposure to 2.7 μM AA. Thus, according to the present results AA is a weak ENA inducer in *A. anguilla* L. However, early increases in EMN and ENA frequency at 2 and 6 h exposure, respectively, to 0.9 and 0.0125 μM AA in juvenile sea bass (Gravato and Santos, 2002a, b) suggest that this juvenile fish species is highly sensitive compared with adult eels. Other research work concerning the Ames test demonstrated that the PME chlorination stage induces mutations in *Salmonella typhimurium* (Kamra et al., 1983) and its mutagenicity decreased with decreasing amounts of free chlorine contained in those liquors resulting from the bleaching process (Nylund et al., 1994). Previous studies with eels exposed to BaP demonstrated that 2.7 μM had a prolonged ENA effect from 72 to 216 h exposure (Maria et al., 2002), whereas BNF had no ENA effect on eels from 2 to 72 h exposure (Teles et al., 2002). An increase in ENA frequency was also observed in *A. anguilla* L. exposed to 25% BKPPME at 8, 16, and 24 h exposure to 25% BKPPME and at 24 h exposure to 50% BKPPME by Maria et al. (2003a), suggesting that the effluent dilution uncovers the ENA-inducing effect of resin acids such as AA.

The results of Pérez-Alzola and Santos (1997) demonstrated the lack of an increase in EMN frequency in Chinese hamster ovary (CHO) cells, after pine kraft bleached effluent or effluent derived from a biobleaching process treatment plant. However, both effluents increased sister chromatid exchange (SCE) frequencies in CHO cells. Elevation of levels of rainbow trout (*Onchorhynchus mykiss*) hepatic micronuclei was demonstrated after injection of a mutagenic fraction from PME extracts, despite the absence of a mutagenic responses using TA 100 without microsomal activation (Rao et al., 1997).

The present genotoxicity results demonstrate that AA is a DNA strand break inducer in eel blood, since high and low AA concentrations promoted early and late decreases in blood DNA integrity in *A. anguilla* L., respectively. Thus, the blood DNA integrity results suggest that low AA concentrations promote late decreases in blood DNA integrity, since it decreased after 24 (14.86%) and 72 (14.71%) h exposure to 0.1 μM AA compared with controls. A decrease in blood DNA integrity was also observed at 24 h (12.48%) exposure to 0.3 μM AA. Nevertheless, high AA concentrations were early blood genotoxic inducers compared with low AA doses, since decreases in blood DNA integrity were detected at 8 (22.33%) h exposure to 0.9 μM AA, as well as at 8 (15.90%) and 16 (15.36%) h exposure to 2.7 μM AA compared with controls. The early genotoxic effect of blood DNA strand breaks, in eels, was also observed at 8, 16, and 24 h exposure to 50% BKPPME and at 16 and 24 h exposure to 25% BKPPME (Maria et al., 2003a). Previous research work concerning BaP exposure demonstrated that high BaP concentrations (0.9 and 2.7 μM) promote early and prolonged increases in blood DNA strand breaks in eels, compared with 0.3 μM BaP (Maria et al., 2002). Exposure of eels to BNF demonstrated that a high BNF concentration (2.7 μM) caused an early and punctual increase in blood DNA breakage compared with 0.9 μM BNF (Maria et al., 2003b).

El Adlouni et al. (1995) did not observe any significant increase in bulky/aromatic DNA adduct levels in white sucker fish (*Catostomus commersoni*) from a site influenced by a pulp and paper mill.

However, recent research studies (Wilson et al., 2001; Ericson and Larsson, 2000) demonstrated that BKME exposure could be a source of genotoxic compounds in the environment and increased hepatic DNA adduct concentration in a dose-dependent manner in *Onchorhynchus tshawytscha* and *Perca fluviatilis*.

According to the present research results concerning exposure of the eels to AA, all concentrations decreased liver DNA integrity, thereby increasing liver DNA damage. Despite the early increases in blood and liver DNA strand breaks observed at 8 h exposure to the highest AA concentrations (0.9 and 2.7 μM), the highest genotoxic potential of AA was observed in *A. anguilla* L. liver. Thus, high AA concentrations such as 0.9 and 2.7 μM AA promoted decreases in liver DNA integrity after 8 (24.66%, 14.42%), 16 (20.01%, 25.99%), 24 (16.06%, 19.34%) and 72 (12.16%, 11.73%) h exposure compared with controls. The highest decrease in liver DNA integrity was observed at 16 (25.99%) h exposure to 0.9 μM AA. Maria et al. (2002, 2003b) previously demonstrated genotoxic effects of BaP and BNF on eels, measured as decreases in liver DNA integrity. Comparison of BaP, BNF, and AA with respect to genotoxic potential in eels, measured as decreases in liver DNA integrity revealed that AA was stronger than BNF and BaP, since 0.3 μM BNF had no genotoxic effect and the same BaP molar concentration promoted derecease in liver DNA integrity only at 144 h exposure (Maria et al., 2002, 2003b).

Maria et al. (2003a) also previously demonstrated decreases in liver DNA integrity in adult *A. anguilla* L. exposed to 25 and 50% BKPPME. However, 25% BKPPME induced liver DNA strand breaks only at 24 h exposure, followed by recovery at 72 h, whereas 50% BKPPME induced prolonged liver DNA damage up to 72 h exposure.

The present hematological results revealed early increases in Hb (8 h) in response to 2.7 μM AA as well as at 16 h in response to 0.9 and 0.3 μM AA. However, a delayed decrease in Hb was observed at 72 h exposure to 0.1, 0.9, and 2.7 μM AA. Furthermore, the Hb/RBC ratio increased at 16 and 24 h exposure to 0.3 and 2.7 μM AA, whereas it decreased at 72 h exposure to 0.9 and 2.7 μM AA. The decrease in Hb/RBC ratio seems to be related to a RBC metabolic failure in Hb synthesis rather than to hemolysis, since the RBC count remained constant.

The direct cause and effect relationship between increased liver EROD activity and liver and blood DNA strand breaks as well as ENAs is not easy to establish, since low liver EROD activity values measured in AA-exposed eels seem capable of inducing early increases in liver DNA strand breaks, whereas ENAs are observed only at the highest AA concentration after a much longer exposure period.

5. Conclusions

AA is more genotoxic to liver than to blood in *A. anguilla* L., as far as DNA strand breaks are concerned. However, AA is a weak ENA inducer in *A. anguilla* L., since only the highest AA concentration caused a delayed increase in ENA frequency. *A. anguilla* L. liver EROD-inducing potency was revealed at 24 h for all AA concentrations, despite its highly significant increase at 0.9 μM AA during the whole experiment. Eel liver GST activity demonstrated that only low AA concentrations promote increases in liver GST, whereas high AA concentrations do not alter it. Liver damage was demonstrated as decreases in ALT at high AA concentrations.

In *A. anguilla* L. exposed to AA the relationship between high hepatic phase I biotransformation and genotoxicity is difficult to establish, since low liver EROD activities seem capable of inducing early increases in liver DNA strand breaks, whereas ENAs were observed only after long exposures to the highest AA concentration.

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