
ACUTE TOXICITY OF DEHYDROABIETIC ACID TO RAINBOW TROUT: MANIPULATION OF BIOTRANSFORMATION ENZYMES

David L. Straus^{*1}, Trevor R. Stuthridge, Sheree M. Anderson and John S. Gifford

New Zealand Forest Research Institute, Pulp and Paper Research Organisation (PAPRO),

Environmental Research Group, Private Bag 3020, Rotorua, New Zealand

¹Current address: Stuttgart National Aquaculture Research Center, USDA/ARS,

P.O. Box 860, Stuttgart, AR, 72160, USA.

Received, 17/12/97; accepted, 23/4/98.

ABSTRACT

Resin acids constitute the most important group of acutely toxic chemicals to fish in waste waters of pulp mills; dehydroabietic acid (DHAA) is one of the most persistent and abundant. The acute toxicity of DHAA has been previously determined in rainbow trout (*Oncorhynchus mykiss*); however, the effect of biotransformation enzyme manipulation on acute toxicity is not known. Piperonyl butoxide (PBO) and salicylamide (SAL) were utilised in the present study to determine their role in the acute toxicity of DHAA and their effects on ethoxyresorufin O-deethylase (EROD), uridine diphospho-glucuronosyltransferase (UDPGT) and glutathione S-transferase specific activities. Estimates of mean 96-h LC50 values were 1.79, 1.58, and 1.80 mg/L DHAA for control, PBO- and SAL-treated fish, respectively; acute toxicity of DHAA in PBO-treated fish was significantly higher than in controls and other treatments. Specific activities indicated that PBO significantly induced EROD response. Unexpectedly, SAL-treated fish did not have an inhibited UDPGT response (substrate = *p*-nitrophenol) despite residue analyses which indicated that SAL inhibited glucuronidation of DHAA. These results suggest that manipulation of glucuronidation (detoxication) by SAL has little or no effect on the acute toxicity of DHAA to rainbow trout. Piperonyl butoxide treatment induces EROD and increases toxicity, suggesting that Phase I biotransformation has an important role in the acute toxicity of DHAA.

A separate experiment studied the effects of β -naphthoflavone (β NF) treatment on biomarker response, to compare relative activities of the biomarkers with the PBO- and SAL-treated fish. Specific activity of EROD was increased 34- (β NF treatment) and 3-fold (PBO treatment) when compared to their respective controls; β NF treatment increased UDPGT response 2-fold. The β NF was a superior inducer, but was not used in the acute toxicity study because the invasive treatment technique could compromise the results.

Key words: biotransformation enzymes, dehydroabietic acid, piperonyl butoxide, rainbow trout, salicylamide

INTRODUCTION

Effluents from pulp mills (kraft, sulphite and mechanical) contain many compounds that have been found to be toxic to aquatic organisms. Resin acids, found in effluents from pulp mills using pine softwoods as feedstock, are an important group of chemicals that are acutely toxic to fish; dehydroabietic acid (DHAA) is one of the most persistent and abundant (Oikari *et al.* 1983). The acute toxicity or LC50 (median lethal concentration) of DHAA has been previously determined for rainbow trout (*Oncorhynchus mykiss*) in several studies (Davis and Hoos 1975; Leach and Thakore 1976; Chung *et al.* 1979). However, the mode-

of-action of DHAA toxicity and the effect of biotransformation enzyme manipulation is not known.

The use of inhibitors or inducers of cytochrome P450 isoforms (P450s) and other biomarkers assists with the mechanistic interpretation of toxicological processes. To fully examine the involvement of specific enzymes in these processes, chemical probes with inhibitory or inductive selectivity can be valuable. Enzyme induction or inhibition can reduce or enhance toxicity depending on the compound involved. Although enzyme manipulation generally does not change the

*Author for correspondence; e-mail: DLS@INAME.COM

References to trade names, commercial products, or manufacturers, do not imply or constitute endorsement or recommendation for use.

DHAA, trout and biotransformation enzymes.

pathways of oxidative metabolism, it does alter the reaction rate and often appears to shift the significant detoxication reactions to non-oxidative mechanisms. Oxidation or hydroxylation reactions can form products of reduced or enhanced potency; thus, enzyme manipulation can increase and/or decrease the toxicity of a chemical, depending on the shift in the balance of the competing activation or detoxication reactions (Casida 1970).

Previous research by several labs (Glickman *et al.* 1977; Melancon *et al.* 1977; Erickson *et al.* 1988; Erickson *et al.* 1992) has incorporated piperonyl butoxide (PBO) in the water column as an inhibitor/inducer of mixed-function oxidase (MFO) enzymes in rainbow trout. Lech (1974) and Lech and Statham (1975) utilised salicylamide (SAL) as an inhibitor of glucuronyltransferase in rainbow trout and sea lamprey (*Petromyzon marinus*). In a recent study by Podowski *et al.* (1991), both of these inhibitors were used to study the biotransformation and disposition of hexachlorocyclopentadiene in goldfish (*Carassius auratus*). However, there is a paucity of information concerning biomarker response of fish exposed to PBO and SAL.

Piperonyl butoxide is a methylenedioxyphenyl compound that has routinely been used, since its introduction in 1947, as a synergist in pesticide formulations because it inhibits MFO activity in insects. There is a similar effect in mammals; however, metabolic degradation of PBO in insects is much slower resulting in more persistent effects (Casida 1970). The synergistic effect of PBO is exerted through competitive inhibition by the parent molecule and non-competitive inhibition through formation of a stable complex between the metabolite(s) and P450s (Hodgson and Philpot 1974; Franklin 1977; Wilkinson *et al.* 1984). The initial inhibition of MFOs by PBO is usually followed by induction within 24 to 48 h in mice (Skrinjaric-Spoljar *et al.* 1971; Fennell *et al.* 1980). Recent research by Grøsvik *et al.* (1997) demonstrates that this biphasic effect occurs in Atlantic salmon (*Salmo salar* L.) as well; in both studies PBO was administered to animals by intraperitoneal (IP) injection.

Treatment with SAL causes a reduction in the capacity to form glutathione or glucuronide conjugates of subsequently administered compounds or their metabolites by saturation of the glucuronide formation process (Sipes and Gandolfi 1991). In humans, this is carried out by the conversion of SAL to SAL-sulfate which causes inhibition of the formation of glucuronides by competition for uridine diphosphoglucuronic acid or for UDP-glucuronyltransferase (Levy and Procknal 1968).

The present study was designed to characterise the role of Phase I and Phase II biotransformation enzymes in rainbow trout that are involved in the acute toxicity of DHAA by incorporating PBO and SAL into the bioassays. The specific activities of the Phase I enzyme ethoxyresorufin O-deethylase (EROD) and the Phase II enzymes uridine diphospho-glucuronosyltransferase (UDPGT) and glutathione S-transferase (GST) were measured to observe the *in vivo* response from these synergists. Further, biomarker specific activities of fish injected with 8-naphthoflavone (8NF) were quantified to compare responses from a known inducer of EROD and UDPGT in fish.

MATERIALS AND METHODS

Chemicals

Dehydroabiatic acid was obtained from Helix Biotechnologies, Richmond, B.C., Canada; the certified purity was 99+%. Piperonyl butoxide was purchased from TCI Chemicals, Tokyo, Japan. Salicylamide, 8NF, and other chemicals were purchased from the Sigma Chemical Co., St. Louis, Missouri, USA.

Animals and Treatments

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from the Ngongotaha Fish Hatchery of the New Zealand Eastern Fish and Game Council (Table 1), and held for several weeks at $12 \pm 1.0^\circ\text{C}$ in a 2400 L recirculating system containing dechlorinated tap water. Fish were fed a commercial, 35% protein, crumbled feed every other day to maintain body weight. Fish were not fed for 48 h prior to treatments or during the studies. All procedures were in accordance with the guidelines of the New Zealand Forest Research Institute Animal Ethics program.

Acute toxicity study

Ninety-six h static toxicity tests (APHA 1992) were conducted in 90 L glass aquaria filled with 80 L of water from the recirculating system; seven fish (av. wt 19 g) were present in each aquarium. Water temperatures were maintained at $12 \pm 1.0^\circ\text{C}$ with a 12-h light/dark cycle; an air-stone in each aquarium maintained dissolved oxygen concentrations greater than 90% saturation. Each tank was treated with a nominal concentration of 1 mg/L PBO or 25 mg/L SAL for 4 h, followed by the addition of nominal concentrations of DHAA that remained for the duration of the experiment. Acetone was used as the carrier for the compounds and in the control tanks at the rate of 0.25 mL/L. There were three replications per treatment; each treatment consisted of a control (acetone, PBO, or SAL only) and four concentrations of DHAA to give from 0% to 100% mortality within 96 h based on the results of

DHAA, trout and biotransformation enzymes.

Table 1. Juvenile rainbow trout wet weight and total length (mean \pm SEM) for each replication in the present studies.

| | Replication 1 ¹ | Replication 2 ¹ | Replication 3 ¹ | BNF Study ² |
|-------------|----------------------------|----------------------------|----------------------------|------------------------|
| Weight (g) | 16.8 \pm 0.61 | 19.0 \pm 1.01 | 20.58 \pm 1.17 | 116.3 \pm 3.89 |
| Length (mm) | 122.0 \pm 1.76 | 127.7 \pm 2.25 | 128.3 \pm 2.05 | 251.7 \pm 16.72 |

¹ Fish from the acute toxicity study. n = 21.

² n = 9.

preliminary experiments. Observations were made every 12 h and dead fish were removed immediately.

β -naphthoflavone study

In a subsequent study in the same static system, fish from the previous year's spawn (av. wt 116 g) were injected IP with either carrier or with 50 mg BNF/kg body weight and sacrificed after 96 h (fish were not exposed to DHAA). Corn oil was used as the vehicle at the rate of 10 mL/kg; there were three fish per treatment.

Tissue samples

At the termination of each assay, control fish were sacrificed and the liver was surgically removed, rinsed with 0.9% NaCl and quickly chilled to decrease enzyme activity. All subsequent steps were carried out at 0 to 4°C. Due to the small size of the trout in the acute toxicity study, several livers were combined for each replication; individual fish in the BNF study were large enough to provide adequate liver sample for biomarker enzyme analyses. To complement the acute toxicity study, chemical analyses of fish exposed to DHAA were undertaken to study partitioning between bile, liver and muscle (Stuthridge *et al.* 1997).

Microsomal fractions were isolated as described in Forsyth and Chambers (1989). Briefly, the livers were placed in 6 mL of 50 mM Tris-HCl + 150 mM KCl buffer (pH 7.4 at 25°C) per gram of tissue, and homogenised with a glass/Teflon motor-driven homogeniser. The microsomal fraction was isolated from liver homogenates by centrifuging a 17 000 \times g (15 min) supernatant fraction at 110 000 \times g for 60 min. All centrifugations were conducted in Sorvall centrifuges. The microsomal pellets were layered with glycerol storage buffer and stored at -80 \pm 1°C until analyses (<1 year).

Enzyme Assays

Microsomal EROD specific activity (pmol \cdot min⁻¹ \cdot mg protein⁻¹) was quantified fluorometrically by a modification of the method of Pohl and Fouts (1980) and Munkittrick *et al.* (1993). Liver microsomes, 100 mg wet weight equivalent, were warmed in 50 mM Tris-HCl (pH 7.8 at 25°C) buffer at 22°C for 10 min in the presence of 10 mM MgSO₄, 2 mg/mL BSA, and a NADPH-generating system to give a final concentration of 5 mM glucose-6-phosphate and 10 mM NADP⁺ plus 0.25 U of glucose-6-phosphate dehydrogenase; the final reaction volume was 1 mL. The reaction was initiated by the addition of 3 M (final concentration) of 7-ethoxyresorufin and incubated at 22°C for 10 min. Reactions were terminated by the addition of 2 mL methanol (to initiate protein precipitation) with continued incubation for 10 min; mixtures were then centrifuged for 10 min at 17 500 \times g. Resorufin content was determined by measuring the fluorescence at 585 nm with excitation at 550 nm in a Perkin-Elmer 204-S fluorescence spectrophotometer.

Specific activity of GST was quantified spectrophotometrically in the cytosolic fraction by the method of Förlin and Haux (1985). The incubation mixture contained 40 μ L of the 110 000 \times g supernatant, 1 mM glutathione, 2 mM 1-chloro-2,4-dinitrobenzene and 100 mM K-phosphate buffer (pH 7.4 at 25°C) in a total volume of 4 mL. The reaction was initiated with the addition of the supernatant and measured for 1 min in a GBC 918 UV-Visible double beam spectrophotometer at 22°C and 344 nm. The extinction coefficient of 9.6 mM⁻¹ \cdot cm⁻¹ was used to calculate specific activity units as μ mol \cdot min⁻¹ \cdot mg protein⁻¹.

Total microsomal P450s content was quantified by the carbon monoxide-difference spectroscopy method of Omura and Sato (1964) in 200 mg wet weight equivalent microsomes; a GBC 918 UV-Visible double

DHAA, trout and biotransformation enzymes.

beam spectrophotometer was used to scan the sample from 400 to 500 nm. An extinction coefficient of $91 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ was used to calculate $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$.

Microsomal UDPGT specific activity was quantified by a modification of the method of Castrén and Oikari (1983). A solution consisting of 150 μL of 50 mM Tris-HCl (pH 7.4 at 25°C) buffer and 100 μL of 500 mM K-phosphate buffer containing 350 mM *p*-nitrophenol, 6.89 mM uridine 5'-diphosphoglucuronic acid (UDPGA), and 10 mM K_2EDTA was warmed at 22°C for 5 min; the blank did not contain UDPGA. The reaction was initiated by the addition of 50 mg wet weight equivalent liver microsomes (50 μL) and incubated at 22°C for 20 min. Reactions were terminated by the addition of 900 μL cold 3% trichloroacetic acid (W/V). The mixture was centrifuged for 10 min at 17 500 $\times g$ to precipitate proteins and the supernatant solution added to 100 μL of 5 M NaOH. Absorbance was measured at 400 nm on a GBC 918 UV-Visible double beam spectrophotometer. The amount of *p*-nitrophenol remaining was calculated using the molar absorption coefficient of $18.8 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ to give specific activity units of $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$.

Determination of protein content

Microsomal and cytosolic protein concentrations were quantified by the method of Lowry *et al.* (1951). Bovine serum albumin was used as the standard.

Statistics

Estimates of LC50 were calculated using log-probit analysis (SAS Institute, Cary, North Carolina, USA) with three replications per data point. The LC50 value means

and biomarker enzyme specific activities were analysed for differences by ANOVA followed by the Student-Neuman-Keuls means comparison test using SAS. A level of $p < 0.05$ was used to conclude a significant difference among means.

RESULTS AND DISCUSSION

Acute toxicity

Convulsions and loss of equilibrium, similar to symptoms observed by Oikari *et al.* (1982), were noticed in fish prior to death in the acute toxicity study. Fish in the present study also contained a large amount of water in their stomachs. There was no mortality in the control tanks.

Estimates of 96-h LC50 are shown in Table 2; the acute toxicity of DHAA in PBO-treated fish was significantly higher than the control or SAL-treated fish. Median lethal concentration values in the control treatment of the present study were similar to estimates previously determined by Leach and Thakore (1976), Davis and Hoos (1975), and Chung *et al.* (1979).

Lech and Statham (1975) demonstrated that SAL treatment increased the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) 2.7-fold in rainbow trout, but did not alter the toxicity of TFM to sea lamprey. Podowski *et al.* (1991) found PBO and SAL treatment did not affect the toxicity of hexachlorocyclopentadiene to goldfish. In the present study, PBO treatment significantly increased toxicity of DHAA 1.1-fold. Differences in toxicity of a chemical when using biotransformation enzyme inducer/inhibitors generally relate to the mode-of-action of the specific class of compound; such toxicity differences can also be species-specific as

Table 2. Estimates of 96-h LC50 (mean \pm SEM) for juvenile rainbow trout of each treatment in the acute toxicity study.

| Treatment | LC50 (mg/L) ¹ |
|--------------------|------------------------------|
| Control | 1.79 ^A \pm 0.02 |
| Piperonyl Butoxide | 1.58 ^B \pm 0.06 |
| Salicylamide | 1.80 ^A \pm 0.04 |

¹ n = 3; LC50 values were calculated by log-probit analysis; means not followed by the same capital letter are significantly different ($p \leq 0.05$) by the Student-Neuman-Keuls means comparison test.

DHAA, trout and biotransformation enzymes.

shown by Lech and Statham (1975). Results of the present acute toxicity study suggest that manipulation of Phase I biotransformation enzymes by treatment with PBO increases toxicity of DHAA, but manipulation of Phase II enzymes by treatment with SAL has no measurable effect on the toxicity of DHAA to rainbow trout.

Biomarker analyses

Biomarker specific activities from the control fish (not exposed to DHAA) of each treatment in the acute toxicity study (Table 3) indicate that PBO treatment significantly induced EROD specific activity 3-fold, while UDPGT and GST specific activities and P450s content were unaffected by the treatments. Fish in the SAL treatment had an apparent inhibition of EROD specific activity, but there were no significant differences due to large variance in microsomal enzyme response. Specific activities for UDPGT and GST have not been quantified in previous studies with PBO or SAL.

The Phase I enzyme EROD is a commonly used biomarker in aquatic species and is mainly catalysed by one isoform of P450, designated cytochrome P4501A, which is efficiently induced by compounds

such as polycyclic aromatic hydrocarbons (PAHs) in fish liver (Stegeman 1989). Cytochrome P4501A is important in studies on the mechanisms of toxicity since this isoform metabolises many PAHs to reactive intermediates in fish (Stegeman 1989). Induction of EROD in the present study by PBO treatment and the consequent lower LC50 value suggests that cytochrome P4501A either directly or indirectly plays an active role in the acute toxicity of DHAA; possibly by forming an unknown reactive intermediate(s). Erickson *et al.* (1992) demonstrated that pretreatment with PBO significantly elevated EROD activity while significantly reducing the rate of oxidation of rotenone in hepatic microsomes of rainbow trout. Such information concerning oxidation rates suggest that DHAA would be more toxic prior to any biotransformation; however, the present study did not include metabolite profiles.

Previous studies have utilised SAL as an inhibitor of glucuronyltransferase (Lech 1974; Lech and Statham 1975; Podowski *et al.* 1991); however, in their studies, UDPGT response was not quantified *in vivo*. Surprisingly, SAL-treated fish of the present study did not have an inhibited UDPGT response. A study by Clarke *et al.* (1991) reported that several isoforms of UDPGT are found in fish, each having different substrate

Table 3. Hepatic biomarker specific activity (mean \pm SEM) from control juvenile rainbow trout (not exposed to DHAA) in the acute toxicity study.

| Parameter | Treatment ^{1,2} | | |
|--|-------------------------------|---------------------------------|-------------------------------|
| | Control (Acetone) | Piperonyl Butoxide | Salicylamide |
| EROD | | | |
| (pmol \cdot min ⁻¹ \cdot mg protein ⁻¹) | 30.96 \pm 6.82 ^A | 106.49 \pm 16.62 ^B | 15.95 \pm 3.83 ^A |
| GST | | | |
| (μ mol \cdot min ⁻¹ \cdot mg protein ⁻¹) | 0.50 \pm 0.04 ^A | 0.49 \pm 0.04 ^A | 0.38 \pm 0.04 ^A |
| P450s | | | |
| (nmol \cdot mg protein ⁻¹) | 0.31 \pm 0.03 ^A | 0.26 \pm 0.01 ^A | 0.36 \pm 0.04 ^A |
| UDPGT | | | |
| (nmol \cdot min ⁻¹ \cdot mg protein ⁻¹) | 0.91 \pm 0.15 ^A | 0.64 \pm 0.10 ^A | 0.77 \pm 0.15 ^A |

¹ Due to fish size, 7 livers were pooled for each microsomal fraction.

² n = 3; means within a row not followed by the same capital letter are significantly different (p \leq 0.05) by the Student-Neuman-Keuls means comparison test.

DHAA, trout and biotransformation enzymes.

specificity. Residue analysis to determine the percent unconjugated DHAA in liver of trout from the present study suggests that SAL significantly inhibited glucuronide formation (Stuthridge *et al.* 1997) compared to the control. A biomarker response for SAL-treated fish was not reflected in UDPGT specific activities, however, SAL may inhibit an isoform of glucuronyltransferase that is not specific for *p*-nitrophenol. Alternately, GST may form some other type of glucuronide (or conjugate) which was not quantified by Stuthridge *et al.* (1997).

The present study was not designed to measure biomarker response in fish exposed to DHAA, however, much of this data has been previously reported. Oikari and Lindström-Seppä (1990) observed that doses of 50 and 200 µg DHAA/L were not responsible for induction of EROD specific activity in juvenile rainbow trout after 17 days. However, Pesonen *et al.* (1993) showed a dose-dependent decrease in EROD specific activity of primary hepatocytes with exposure to DHAA, and suggested that this resin acid may directly affect the cytochrome P450 enzyme system or interfere with the lipid structure of the endoplasmic reticulum or other factors important for the catalytic activity of P450s. The UDPGT specific activity was strongly decreased in liver and kidney of rainbow trout exposed to a sub-lethal concentration of DHAA which averaged 1.2 mg/L over 4 days (Oikari *et al.* 1983). Another study by Oikari *et al.* (1984) suggested that glucuronidation was a major pathway for elimination of resin acids.

Oikari and Lindström-Seppä (1990) and Munkittrick *et al.* (1993) have reported that pulp and paper effluents have been found to contain compounds that induce cytochrome P4501A, as measured by EROD specific activity. The results of the present study demonstrate that induced EROD specific activity is consistent with increased acute toxicity of DHAA, a major toxicant of many softwood pulp and paper effluents. Therefore, measuring EROD is an important parameter for assessing the biological impacts of pulp and paper effluents.

In a separate study, the effect of ßNF treatment on biomarker specific activity (Table 4) showed a significant induction of EROD and UDPGT after 96 h. Zhang *et al.* (1990) reported similar results by three days after injection with 50 mg ßNF/kg; significant induction of P450s by three days and of GST by 14 days were also reported. The ßNF experiment was carried out to compare relative activities of the PBO, SAL, and ßNF treatments with their respective controls (Table 5); fish of the ßNF treatment were markedly higher for P450s, EROD, and UDPGT (2.3-, 9.9-, and 2.6-fold, respectively) than fish of the PBO treatment.

Enzyme induction by ßNF was not included in the present acute toxicity study because the invasive treatment (IP injection) could confound mortalities caused by DHAA.

Previous studies have shown relative induction of EROD activity by PBO (Erickson *et al.* 1988) and by ßNF or PBO (Erickson *et al.* 1992) to be similar to responses of the present study. Relative biomarker response shows that ßNF was a 10-fold better inducer of EROD than PBO in the present studies; however, caution must be used when observing these responses because they were not obtained from a single study. Förlin (1980) studied age related monooxygenase activity and total P450s content in rainbow trout; data demonstrates a slight increase, however, statistics for control fish were not included and data suggest that the differences were not significant. Fish of the present studies were younger than those observed by Förlin (1980).

In summary, results suggest that cytochrome P4501A has an important role in the toxicity of DHAA. Glucuronidation by the isozyme specific for *p*-nitrophenol does not affect inherent toxicity of the compound. Future experiments should incorporate PBO and other non-invasive synergists when observing biomarker response as it relates to acute toxicity; molecular biology techniques should also be utilised when possible. This may help to discover a biotransformation enzyme biomarker (partially) responsible for the acute toxicity of a compound. Further research is also needed to observe the dose-response and time-course of inhibition/induction by PBO administered via the water column in fish, to obtain metabolite profiles of DHAA in fish (including the use of inducers/inhibitors) and to evaluate the inhibitory mode of action of SAL.

ACKNOWLEDGEMENTS

This manuscript was improved by comments from Drs Russell Carr, Chris Hickey, and Steven Levine. Supply of rainbow trout by the New Zealand Eastern Fish and Game Council is greatly appreciated. PAPRO Technical Report No. C621, June, 1996.

DHAA, trout and biotransformation enzymes.

Table 4. Hepatic biomarker specific activity (mean \pm SEM) from control and β -naphthoflavone-treated juvenile rainbow trout in the β NF study.

| Parameter | Treatment ¹ | |
|--|------------------------------|---------------------------------|
| | Control | β -naphthoflavone |
| EROD | | |
| (pmol \cdot min ⁻¹ \cdot mg protein ⁻¹) | 7.01 \pm 3.56 ^A | 237.58 \pm 32.11 ^B |
| GST | | |
| (μ mol \cdot min ⁻¹ \cdot mg protein ⁻¹) | 0.19 \pm 0.01 ^A | 0.14 \pm 0.01 ^A |
| P450s | | |
| (nmol \cdot mg protein ⁻¹) | 0.24 \pm 0.01 ^A | 0.47 \pm 0.08 ^A |
| UDPGT | | |
| (nmol \cdot min ⁻¹ \cdot mg protein ⁻¹) | 0.53 \pm 0.10 ^A | 0.95 \pm 0.04 ^B |

¹ n = 3; means within a row not followed by the same capital letter are significantly different (p \leq 0.05) by the Student-Neuman-Keuls means comparison test.

Table 5. Mean biomarker response of juvenile rainbow trout from separate experiments within the present study of piperonyl butoxide-, salicylamide-, and β -naphthoflavone-treated control fish relative to respective control fish. Fish for the β NF study were from the previous year's spawn; see Table 1 for fish weight and length.

| Parameter | Treatment | | |
|--------------|--------------------|--------------|-------------------------|
| | Piperonyl Butoxide | Salicylamide | β -naphthoflavone |
| EROD | 344% | 52% | 3389% |
| GST | 98% | 76% | 74% |
| P450s | 84% | 116% | 196% |
| UDPGT | 70% | 85% | 179% |

DHAA, trout and biotransformation enzymes.

REFERENCES

- APHA (American Public Health Association), American Water Works Association and Water Pollution Control Federation. 1992. *Standard Methods for the Examination of Water and Wastewater, Part 8000 - Toxicity*, 17th ed. New York, New York, USA, pp 8.1-8.22.
- Casida JE. 1970. Mixed-function oxidase involvement in the biochemistry of insecticide synergists. *J. Agric. Food Chem.* **18**, 753-772.
- Castrén M and Oikari A. 1983. Optimal assay conditions for liver UDP-glucuronosyltransferase from the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **76C**, 365-369.
- Chung LTK, Meier HP and Leach JM. 1979. Can pulp mill effluent toxicity be estimated from chemical analysis? *TAPPI*. **62**, 71-74.
- Clarke DJ, George SG and Burchell B. 1991. Glucuronidation in fish. *Aquat. Toxicol.* **20**, 35-56.
- Davis JC and Hoos RAW. 1975. Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays. *J. Fish. Res. Board Can.* **32**, 411-416.
- Erickson DA, Goodrich MS and Lech JJ. 1988. The effect of piperonyl butoxide on hepatic cytochrome P-450-dependent monooxygenase activities in rainbow trout (*Salmo gairdneri*). *Toxicol. Appl. Pharmacol.* **94**, 1-10.
- Erickson DA, Laib FE and Lech JJ. 1992. Biotransformation of rotenone by hepatic microsomes following pretreatment of rainbow trout with inducers of cytochrome P450. *Pestic. Biochem. Physiol.* **42**, 140-150.
- Fennell TR, Sweatman BC and Bridges JW. 1980. The induction of hepatic cytochrome P-450 in C57 BL/10 and DBA/2 mice by isosafrole and piperonyl butoxide. A comparative study with other inducing agents. *Chem. Biol. Interact.* **31**, 189-201.
- Förlin L. 1985. Effects of clophen A50, 3-methylcholanthrene, pregnenolone-16 α -carbonitrile, and phenobarbital on the hepatic microsomal cytochrome P-450-dependent monooxygenase system in rainbow trout, *Salmo gairdneri*, of different age and sex. *Toxicol. Appl. Pharmacol.* **54**, 420-430.
- Förlin L and Haux C. 1985. Increased excretion in the bile of 17 β -[3H]estradiol-derived radioactivity in rainbow trout treated with β -naphthoflavone. *Aquat. Toxicol.* **6**, 197-208.
- Forsyth CS and Chambers JE. 1989. Activation and degradation of the phosphorothionate insecticides parathion and EPN by rat brain. *Biochem. Pharmacol.* **38**, 1597-1603.
- Franklin MR. 1977. Inhibition of mixed-function oxidations by substrates forming reduced cytochrome P-450 metabolic-intermediate complexes. *Pharmacol. Ther.* **2**, 227-245.
- Glickman AH, Stratham CN, Wu A and Lech JJ. 1977. Studies on the uptake, metabolism, and disposition of pentachlorophenol and pentachloroanisole in rainbow trout. *Toxicol. Appl. Pharmacol.* **41**, 649-658.
- Grøsvik BE, Larsen HE and Goksøyr A. 1997. Effects of piperonyl butoxide and β -naphthoflavone on cytochrome P4501A expression and activity in Atlantic Salmon (*Salmo salar* L.) *Environ. Toxicol. Chem.* **16**, 415-423.
- Hodgson E and Philpot RM. 1974. Interaction of methylenedioxyphenyl (1,3-benzodioxole) compounds with enzymes and their effects on mammals. *Drug Metab. Rev.* **3**, 231-301.
- Leach JM and Thakore AN. 1976. Toxic constituents in mechanical pulping effluents. *TAPPI*. **59**, 129-132.
- Lech JJ. 1974. Glucuronide formation in rainbow trout - effect of salicylamide on the acute toxicity, conjugation and excretion of 3-trifluoromethyl-4-nitrophenol. *Biochem. Pharmacol.* **23**, 2403-2410.
- Lech JJ and Statham CN. 1975. Role of glucuronide formation in the selective toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) for the sea lamprey: comparative aspects of TFM uptake and conjugation in sea lamprey and rainbow trout. *Toxicol. Appl. Pharmacol.* **31**, 150-158.
- Levy G and Procknal JA. 1968. Drug biotransformation interactions in man I. Mutual inhibition in glucuronide formation of salicylic acid and salicylamide in man. *J. Pharm. Sci.* **57**, 1330-1335.
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.* **193**, 265-275.

DHAA, trout and biotransformation enzymes.

- Melancon MJ, Jr, Saybolt J and Lech JJ. 1977. Effect of piperonyl butoxide on disposition of di-2-ethylhexyl phthalate by rainbow trout. *Xenobiotica*. 7, 633-640.
- Munkittrick KR, van den Heuvel MR, Metner DA, Lockhart WL and Stegeman JJ. 1993. Interlaboratory comparison and optimization of hepatic microsomal ethoxyresorufin O-deethylase activity in white sucker (*Catostomus commersoni*) exposed to bleached kraft pulp mill effluent. *Environ. Toxicol. Chem.* 12, 1273-1282.
- Oikari A, Holmbom B and Bister H. 1982. Uptake of resin acids into tissues of trout (*Salmo gairdneri*). *Ann. Zool. Fennici.* 19, 61-64.
- Oikari A, Lönn B-E, Castrén M, Nakari T, Snickars-Nikinmaa B, Bister H and Virtanen E. 1983. Toxicological effects of dehydroabietic acid (DHAA) on the trout, *Salmo gairdneri* Richardson, in fresh water. *Water Res.* 17, 81-89.
- Oikari A, Nakari T and Holmbom B. 1984. Sublethal actions of simulated kraft pulp mill effluents (KME) in *Salmo gairdneri*: residues of toxicants, and effects on blood and liver. *Ann. Zool. Fennici.* 21, 45-53.
- Oikari A and Lindström-Seppä P. 1990. Responses of biotransformation enzymes in fish liver: experiments with pulpmill effluents and their components. *Chemosphere* 20, 1079-1085.
- Omura T and Sato R. 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* 239, 2370-2378.
- Pesonen M, Andersson T and Ahokas J. 1993. The use of primary fish hepatocyte cultures in toxicity studies of paper mill effluents. In *Proceedings of the 47th Appita Annual General Conference*. 2, 811-814.
- Podowski AA, Sclove SL, Pilipowicz A and Khan MAQ. 1991. Biotransformation and disposition of hexachlorocyclopentadiene in fish. *Arch. Environ. Contam. Toxicol.* 20, 488-496.
- Pohl RJ and Fouts JR. 1980. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.* 107, 150-155.
- Sipes IG and Gandolfi AJ. 1991. Biotransformation of toxicants. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 4th ed., Amdur MO, Doull J and Klaassen CD. (Eds), Pergamon Press, New York, USA, pp 88-126.
- Skrinjaric-Spoljar M, Matthews HB, Engel JL and Casida JE. 1971. Response of hepatic microsomal mixed-function oxidases to various types of insecticide chemical synergists administered to mice. *Biochem. Pharmacol.* 20, 1607-1618.
- Stegeman JJ. 1989. Cytochrome P-450 forms in fish: catalytic, immunological and sequence similarities. *Xenobiotica* 19, 1093-1110.
- Stuthridge TR, Anderson SM, Gifford JS, Robinson MJ and Straus DL. 1997. Bioaccumulation of dehydroabietic acid in rainbow trout (*Oncorhynchus mykiss*): Role of biotransformation enzymes. *Wat. Sci. Tech.* 35, 365-372.
- Wilkinson CF, Murray M and Marcus CB. 1984. Interactions of methylenedioxyphenyl compounds with cytochrome P-450 and effects on microsomal oxidation. *Rev. Biochem. Toxicol.* 6, 27-63.
- Zhang YS, Andersson T and Förlin L. 1990. Induction of hepatic xenobiotic biotransformation enzymes in rainbow trout by β -naphthoflavone. Time-course studies. *Comp. Biochem. Physiol.* 95B, 247-253.
-