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Influence of Cytochrome P450 Mixed-Function Oxidase Induction on the Acute Toxicity to Rainbow Trout (Salmo gairdneri) of Primary Aromatic Amines

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The influence of enzyme induction on the acute toxicity of aniline and 4-chloroaniline to rainbow trout (Salmo gairdneri) was investigated. For these two xenobiotics, bioactivation reactions are known to occur in mammals. Induction of cytochrome P450 mixed-function oxidase was obtained by intraperitoneal (ip) injection of trout with a mixture of polychlorinated biphenyls (Aroclor 1254). Five days after ip injection with three different doses of Aroclor 1254 (50, 100, and 200 mg/kg), benz[a]pyrene hydroxylase activity in trout liver microsomes increased five- to sixfold. Cytochrome P450 concentrations in the microsomes were slightly, but significantly, enhanced in two of the three dose levels. The 96-hr LC₅₀'s of aniline and 4-chloroaniline were not affected by pretreatment with Aroclor 1254, suggesting that metabolic activation does not necessarily play a role in the acute toxicity of aromatic amines to fish.

INTRODUCTION

Several research groups have reported that the acute toxicity of certain primary aromatic amines (aniline derivatives that are weakly basic, with octanol-water partition coefficients (log P's) less than 2.9) (Veith and Broderius, 1987; Hermens et al., 1984) is significantly greater than that predicted from baseline narcosis models (e.g., Veith et al., 1983; Könemann, 1981). This increased toxicity has been attributed to two possible explanations. Veith and Broderius (1987) and Bradbury et al. (1989) have proposed that these primary aromatic amines are acting under a more potent narcosis mechanism termed polar narcosis or type II narcosis. Alternatively, Hermens et al. (1984) have postulated that the acute toxicity of these compounds may be a result of metabolic activation to arylhydroxylamine derivatives that elicit acute effects either as an outcome of methemoglobinemia or reactive toxicity.

In mammals, primary aromatic amine-induced methemoglobinemia and chronic toxicity, including carcinogenesis, are clearly a function of enzymatic N-oxidation to hydroxylamine derivatives (Bus and Popp, 1987; Beland and Kadlubar, 1985; Nelson, 1985; Kiese, 1974). Activation of aromatic amines, via N-oxidation, can be catalyzed by cytochrome P450 (Hammons et al., 1985; Gorrod, 1973; Uehleke, 1971), a flavin containing monooxygenase (Hammons et al., 1985; Ziegler, 1980), and prostaglandin H synthase (Wise et al., 1984). It seems that cytochrome P450 is largely re-
sponsible for the N-oxidation of primary aromatic amines, with isoenzymes in the
P448 group having the highest activity (Hammons et al., 1985; Yamazoe et al., 1984; Damani, 1982).

The metabolism of xenobiotics in fish species has been increasingly defined and it
now seems that these organisms can perform many of the same Phase I and Phase II
reactions as mammalian species can, but usually at lower rates (Binder et al., 1984; Gregus et al., 1983). Fish possess a hepatic mixed-function oxidase (mfo) system that
predominately demonstrates activity associated with the P448 group of isoenzymes
(Stegeman et al., 1986; Lech et al., 1982; Stegeman et al., 1981; Franklin et al., 1980;
Statham et al., 1978).

Although there are no reports in the literature of microsomal aromatic N-oxidation
activity in fish, we have recently confirmed the presence of aniline and 4-chloroani-
line N-oxidation activity from hepatic rainbow trout (Salmo gairdnerii) and medaka
(Oryzias latipes) preparations (Bradbury and Dady, unpublished data). If metabolic
activation to N-hydroxy derivatives is central to the acute toxicity of aniline-related
compounds in fish, it is reasonable to assume that induction of cytochrome P450
mixed-function oxidase could enhance the lethality of these chemicals by increasing
the production of activated metabolites. In an effort to evaluate the possible role of
metabolic activation in primary aromatic amine toxicity, we assessed the influence
of enzyme induction, using Aroclor 1254 as an inducer, on the acute lethality of
aniline and 4-chloroaniline to rainbow trout.

MATERIALS AND METHODS

To evaluate the influence of enzyme induction on the acute toxicity of aniline and
4-chloroaniline to rainbow trout, Aroclor 1254 was used as an inducing agent. The
proper dose level of Aroclor 1254 was determined by assessing liver microsomal
benzo[a]pyrene (B[a]P) hydroxylase activity and cytochrome P450 content in pre-
liminary studies. Ninety-six-hour flow-through toxicity tests were then performed
with both induced and noninduced trout.

Chemicals

All buffer components were purchased from Sigma Chemical Co. (St. Louis, MO)
except for potassium hydrophosphate and glycerol, which were purchased from
Fisher Scientific Co. (Eden Prairie, MN). Glucose-6-phosphate, glucose-6-phosphate
dehydrogenase, and NADP were obtained from Sigma Chemical Co. Sodium hydro-
sulfite was obtained from Fisher Scientific Co. 3-Hydroxy B[a]P and B[a]P were ob-
tained from the National Cancer Institute’s Chemical Carcinogen Repository
(Bethesda, MD). Aroclor 1254 (electrical grade) was obtained from Monsanto Chem-
ical Co. (St. Louis, MO). Aniline (purity 99%) and 4-chloroaniline (purity 98%) were
purchased from Aldrich Chemical Co. (Milwaukee, WI).

Isolation of Microsomes and Enzyme Assays

Trout were killed with a blow to the head and the livers were rapidly excised,
chopped with a pair of scissors, and washed in cold KCl (0.154 M). All further steps
were carried out at a temperature < 4°C. The procedure for isolation of the micro-
somes differs slightly from a procedure described by Guengerich (1982). The tissue
was then homogenized in 4 vol KCl (0.154 M) in a Potter-Elvehjem homogenizer. The homogenates were centrifuged for 20 min at 10,000g, while the resulting supernatant was centrifuged at 105,000g for 60 min. The pellet was resuspended in 4 vol 0.1 M phosphate buffer (pH 7.4; 1 mM EDTA) and centrifuged again for 60 min at 105,000g. The final pellet was resuspended in 2 vol of 10 mM Tris buffer (pH 7.4), which contained 1 mM EDTA and 20% glycerol, resulting in microsomal suspensions corresponding to about 0.5 g wet wt liver/ml (4-9 mg protein/ml). The suspensions were stored in a freezer at −80°C for at most 14 days. Storage of the microsomes for up to 14 days had no effect on cytochrome P450 nor B[a]P hydroxylase activity (data not shown). This finding is in agreement with observations of Forlín and Andersson (1985), who observed that storage of microsomes at −80°C in buffer containing 20% glycerol for 1 year did not affect cytochrome P450, ethoxycoumarin-O-deethylase (ECOD) activity, and UDP glucuronyl transferase activities.

Protein content of the microsomes was measured in duplicate using a protein assay kit from Sigma Chemical Co. (No. P 5656), with bovine serum albumin as a standard. Cytochrome P450 content was measured in duplicate according to the procedure described by Omura and Sato (1964). The microsomes were diluted five or seven times in 0.1 phosphate buffer (pH 7.4; 1 mM EDTA; 0.4% Triton X-100). A few crystals of sodium dithionite were added and the baseline absorbance was scanned from 400 to 500 nm. Carbon monoxide was added for about 1 min and the scan was repeated. Cytochrome P450 content (nmol/ml) was calculated from the difference in absorbance at 450 and 490 nm, using an extinction coefficient of 11 mM⁻¹ cm⁻¹.

The B[a]P hydroxylase assay, used in the present study, was based on the procedure described by Nebert and Gelboin (1968). The incubation was carried out in a 0.05 M phosphate buffer (pH 7.4; 5 mM MgCl₂) at 25.0°C (range 24.5–25.5). The incubation mixture (volume 1.0 ml) contained 0.50 pmol NADP, 1 unit glucose-6-phosphate dehydrogenase, 10.0 μmol glucose-6-phosphate, 25.0 nmol B[a]P, and 0.40–1.00 mg protein. B[a]P in an acetone stock solution was added to the incubation mixture in 25 μl aliquots. The reaction was started by adding the glucose-6-phosphate. The reaction was stopped after 10 min by adding 1.0 ml of cold acetone. Hexane (3.0 ml) was added to the incubation tubes and the mixture was shaken for about 1 min by vortex mixing. To 2.0 ml of the upper layer, 4.0 ml of a 1.0 M NaOH solution was added and the mixture was shaken by vortex mixing. The concentration of hydroxylated B[a]P in the alkali phase was determined spectrophotofluorometrically, with excitation at 396 nm and emission at 522 nm. Solutions of 3-hydroxy B[a]P in 0.10 M NaOH were used as standards. The assays were carried out in duplicate and with one blank to which acetone was added prior to the incubation. The incubation and extraction procedures as well as the preparation of the standards were performed in the dark because of the instability of B[a]P and the 3-hydroxy B[a]P.

The optimization studies of the assay were carried out with the same batch of microsomes. It should be noted that the activities given for the induced trout are not specific activities because the assay was only optimized for the noninduced fish.

**Treatment of Rainbow Trout with Aroclor 1254**

Details on origin and holding conditions of the rainbow trout as well as details on water chemistry are given in the subsequent section. The weight of the fish varied from 30 to 120 g. Prior to the dosing with Aroclor 1254, the fish were acclimated for
TABLE I

WATER CHARACTERISTICS DURING 96-hr LC_{50} TESTS WITH RAINBOW TROUT

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>SD (n)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen/DO (mg/liter)</td>
<td>8.5</td>
<td>1.4 (36)</td>
<td>6.2–10.9</td>
</tr>
<tr>
<td>Hardness (mg/liter as CaCO₃)</td>
<td>46.2</td>
<td>1.1 (18)</td>
<td>44.8–48.0</td>
</tr>
<tr>
<td>Alkalinity (mg/liter as CaCO₃)</td>
<td>48.2</td>
<td>6.9 (18)</td>
<td>43.1–66.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.17</td>
<td>0.12 (36)</td>
<td>7.0–7.5</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10.5</td>
<td>0.3 (30)</td>
<td>10.2–11.4</td>
</tr>
</tbody>
</table>

4 days at 11.0 to 11.5°C in stainless-steel tanks. Fish received single intraperitoneal injections (ip) with 0, 50, 100, or 200 mg/kg Aroclor 1254 dissolved in corn oil (1 μl/g fish) and were kept at 11.0 to 11.5°C. Controls received corn oil only. After 5 days the fish were killed by a blow to the head and the liver was removed for analysis of cytochrome P450 content and B[a]P hydroxylase activity. Four samples (one or two fish) were taken from each Aroclor dose group. Preliminary studies showed that ip injection with corn oil did not affect cytochrome P450 content or B[a]P hydroxylase activity (data not shown).

4C_{50} Tests with Rainbow Trout

Rainbow trout were obtained from a local hatchery (Seven Pines Trout Hatchery, Lewis, WI), were held in 530-liter fiberglass holding tanks at 11 to 13°C, and were fed with a commercial dry food. The fish were acclimated for at least 2 weeks prior to testing. The induced and noninduced rainbow trout for the LC_{50} tests were always taken from the same stock. Mean total weight of 10 fish in the control tanks from all six LC_{50} tests were 638 ± 71 g.

The 96-hr LC_{50} tests were carried out in a proportional diluter (Mount and Brungs, 1967) with a dilution factor of 0.6. Exposure tanks were glass aquaria with a volume of 40 liters. Five concentrations of the test chemical and one blank were tested in duplicate. Each tank contained 10 fish. All test water was obtained from Lake Superior. Temperature was measured each day in the control tank. Dissolved oxygen (DO) was measured on one complete set of duplicate tanks. During each test, hardness, alkalinity, and pH determinations (APHA, 1980) were made, at a minimum, on a control, one intermediate, and one high-concentration tank. Data for all water characteristics are summarized in Table 1.

Aniline and 4-chloroaniline were dissolved in Lake Superior water and a predetermined portion of each solution was metered into the diluter during each diluter cycle. The concentrations of the test chemicals in water were analyzed daily during all 96-hr exposures. Water samples for chemical analysis were taken from one complete set of duplicate tanks. Aniline and 4-chloroaniline analyses were accomplished using direct aqueous injection gas chromatography (GC) with flame ionization detection (FID). A Hewlett-Packard Model 5730A GC, equipped with FID, Model 7671A autosampler and linked to a Hewlett-Packard Model 3357 laboratory automation system, was used. A 2 mm i.d. × 1.8 m glass Tenax GC (60–80 mesh) packed column was employed to effect separation. Injection port and detector temperatures were 250 and 300°C, respectively. Nitrogen carrier gas flow was 30 ml/min and peaks were
symmetrical. Specific instrument conditions for aniline were: isothermal oven temperature, 220°C; attenuation, 16×; retention time, 1.35 min and detection limit with a 1-μL injection 0.5 μg/ml. Specific conditions for 4-chloroaniline were: isothermal oven temperature, 210°C; attenuation, 4×; retention time, 1.35 minute; and detection limit with a 2.5-μL injection volume, 0.5 μg/ml. Percentage spike recoveries for aniline were 96.3 ± 4.1 (n = 4) and 102.5 ± 1.5 (n = 4), in LC₅₀ tests with noninduced and induced fish, respectively. For 4-chloroaniline, spike recoveries were 98.2 ± 1.8% (n = 4) and 103.7 ± 9.6% (n = 4).

Data and Statistical Analyses

Kinetic constants for B[a]P hydroxylase activity (Kₘ and Vₘₐₓ) were calculated by a curve-fitting program (University of Utrecht, unpublished method) for Michaelis–Menten kinetics. Testing of statistical differences were performed by analysis of variance, Student’s t-test, or Wilcoxon rank sum test (Snedecor and Cochran, 1967). LC₅₀ values were calculated by the trimmed Spearman–Karber method (Hamilton et al., 1977).

RESULTS

Optimization of B[a]P Hydroxylase Assay

The dependency of B[a]P hydroxylase activity on substrate concentration, protein concentration, and incubation time are given in Figs. 1 and 2. The activity is linearly dependent on protein concentration up to about 0.75–1.0 mg/ml, and on incubation time for up to 10 min (Figs. 2a and 2b). Fitting the data from Fig. 1 with Michaelis–Menten kinetics yields apparent Kₘ and Vₘₐₓ values of 1.63 μM and 0.0140 nmol/min-mg protein, respectively. In the calculation of Kₘ and Vₘₐₓ, the activity at 100 μM B[a]P was not included because the activity was obviously lower at this high substrate concentration.

On the basis of these results, the following standard conditions for the assay were chosen: concentration B[a]P, 25 μM; incubation time, 10 min, and protein concentration, 0.40–0.75 mg/ml.
Cytochrome P450 induction: Toxicity to Rainbow Trout

FIG. 2. Influence of incubation time (a) and microsomal protein (b) concentration on liver microsomal B[a]P hydroxylase activity in rainbow trout. Assay conditions: concentration B[a]P, 25 μM (a and b); concentration of microsomal protein, 0.75 mg/ml (a); incubation time, 10 min (b).

Enzyme Induction Studies

Cytochrome P450 concentrations and B[a]P hydroxylation activities from the Aroclor 1254-treated groups were compared to those of a group that received only corn oil (Table 2). Cytochrome P450 concentrations in the controls were 0.20 ± 0.02 nmol/mg protein. Literature values for cytochrome P450 content of rainbow trout liver microsomes vary from about 0.16 to 0.30 nmol/mg protein (Kleinow et al., 1986; Förlin and Andersson, 1985; Elcombe and Lech, 1979; Förlin, 1980).

TABLE 2

<table>
<thead>
<tr>
<th>Pretreatment (ip injection)</th>
<th>Cytochrome P450 (nmol/mg protein) mean ± SD (n)</th>
<th>B[a]P hydroxylase activity (nmol/min-mg protein) mean ± SD (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil (control)</td>
<td>0.20 ± 0.02 (4)</td>
<td>0.0257 ± 0.0075 (4)</td>
</tr>
<tr>
<td>50 mg/kg Aroclor 1254</td>
<td>0.29 ± 0.03 (4)</td>
<td>0.150 ± 0.039 (4)</td>
</tr>
<tr>
<td>100 mg/kg Aroclor 1254</td>
<td>0.29 ± 0.10 (4)</td>
<td>0.124 ± 0.068 (4)</td>
</tr>
<tr>
<td>200 mg/kg Aroclor 1254</td>
<td>0.34 ± 0.03 (4)</td>
<td>0.163 ± 0.042 (4)</td>
</tr>
</tbody>
</table>

"After ip injection, the trout were kept for 5 days at 10°C before the microsomes were prepared. From each group that received a different dose, four different samples of microsomes were prepared from one liver or pooled livers from two trout.

"Conditions during assay: concentration B[a]P, 25 μM; incubation time, 10 min; concentration of microsomal protein, 0.43–0.74 mg/ml.

* Statistically different from control group at P < 0.05.
TABLE 3

ACUTE TOXICITY OF ANILINE AND 4-CHLOROANILINE IN NONINDUCED AND INDUCED RAINBOW TROUT

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Noninduced trout</th>
<th>Induced trout&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>33.5 (30.0–37.7)</td>
<td>31.6 (29.1–34.3)</td>
</tr>
<tr>
<td>4-Chloroaniline</td>
<td>11.0 (9.7–12.5)</td>
<td>14.0 (13.0–15.1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Induced trout received 50 mg/kg Aroclor 1254 5 days prior to exposure to aniline or 4-chloroaniline.

All groups of trout that were injected with Aroclor 1254 exhibited a five- to sixfold increase in B[a]P hydroxylase activity. The increase in activity, however, was not dose dependent. Cytochrome P450 levels were significantly higher (P < 0.05) in the rainbow trout that received 50 and 200 mg Aroclor 1254/kg but not in the group that was injected with 100 mg/kg, probably due to the relatively high variance in the cytochrome P450 levels of this particular group.

Because the degrees of induction at all three doses were almost similar, the lowest dose of 50 mg/kg was selected for the LC<sub>50</sub> tests.

LC<sub>50</sub> Tests with Rainbow Trout

Values for the 96-hr LC<sub>50</sub>'s of aniline and 4-chloroaniline are given in Table 3. The LC<sub>50</sub>'s for the noninduced and induced fish do not show any significant differences. Aniline and 4-chloroaniline 96-hr LC<sub>50</sub> values were between 33.5 to 31.6 and 11.0 to 14.0 mg/liter, respectively.

DISCUSSION

Inducing agents can be separated into two broad classes based on differential effects on the forms of the terminal oxidase in mfo systems, e.g., cytochrome P450 and cytochrome P448 (Lech et al., 1982). Recently, more forms have been described (Nebert and Gonzalez, 1987). In mammalian mfo systems, phenobarbital (PB) exposure is associated with cytochrome P450 induction (e.g., as measured by benzphetamine-N-demethylase and ethylmorphine-N-demethylase activity), while 3-methylcholanthrene (3-MC) selectively induces cytochrome P448 (e.g., as measured by ethoxyresorufin-O-deethylase (EROD) activity) (Lech et al., 1982). B[a]P hydroxylase, or more generally, arylhydrocarbon hydroxylase, in mammals can be induced by both classes of inducers (Lech et al., 1982).

Studies of enzyme induction in the rat have shown that PCBs have characteristics of both PB and 3-MC. Coplanar PCBs, e.g., 3,4,3',4' PCB, have been described as 3-MC type inducers in mammals (Goldstein et al., 1977) and fish (Lech et al., 1982) because they increase EROD activity. Biphenyls chlorinated symmetrically in both meta and para positions increase the formation of cytochrome P448 and arylhydrocarbon hydroxylase activity, but decrease aminopyrene N-demethylase activity (Goldstein et al., 1977).
Induction of enzyme activities from the mfo system by PCBs has been studied in different species of fish (Andersson et al., 1985; Ankley et al., 1986; Elcombe and Lech, 1979; Förlin, 1980; Lech et al., 1982; Lidman et al., 1976; Melancon and Lech, 1983; Sivarajah et al., 1978). Induction in fish species seems to be different from that in mammals. Specific PB-type inducers in rodents are ineffective as inducers in fish (Lech et al., 1982). In rainbow trout, Aroclor 1254 is a potent inducer of EROD activity (a specific 3-MC-associated activity in rats) and arylhydrocarbon hydroxylase activity (mixed type of associated activity in rats), while no induction is observed for specific PB-linked activities like ethylmorphine-N-demethylase activity (Elcombe and Lech, 1979). From this particular study, and others, Lech et al. (1982) concluded that fish do not respond to PB-type inducers but that most species of fish respond well to 3-MC-type inducers. From these above-mentioned studies it can be concluded that, although the B[a]P hydroxylase assay used in this particular study is not related to one particular type of induction, the observed enhanced activities in our study are likely to be related to the 3-MC type of induction. This is supported by the results of another study where ip injection of rainbow trout with Aroclor 1254 (dose 150 mg/kg) showed induction of EROD activity, which is a more specific 3-MC effect (Lech et al., 1982).

Increase in B[a]P hydroxylase activity after treatment with PCB mixtures, as observed in our study, is well known. Förlin (1980) found an increase in activity of 8.5 to 23 times in rainbow trout 14 days after a single ip injection with Clophen A 50 (500 mg/kg). Cytochrome P450 levels were a factor of 2 higher (significantly different from control). Melancon and Lech (1983) observed higher activities for B[a]P hydroxylase, EROD, and ECOD 5 days after an ip injection of rainbow trout with several doses of Aroclor 1254 (from 0.025 to 200 mg/kg). The highest increase for EROD and ECOD was observed at a dose of 50 mg/kg. Elcombe and Lech (1979) observed higher activities for B[a]P hydroxylase after injection of rainbow trout with several doses of Aroclor 1242 (50, 70, 100, 150, and 200 mg/kg). The highest increase in activity was observed at a dose of 100 to 200 mg/kg. Cytochrome P450 levels of the test groups were not enhanced compared to those of the control group.

In addition to B[a]P hydroxylase activity, cytochrome P450 levels were also increased after pretreatment with Aroclor 1254 (Table 2). It is questionable whether such an increase in cytochrome P450 is an important datum. Elcombe and Lech (1979) observed a small increase in cytochrome P450 (from 0.23 to 0.26 nmol/mg protein), while B[a]P hydroxylase activity was increased from 0.022 to 0.23 nmol/min-mg protein after ip injection of rainbow trout with Aroclor 1242 (150 mg/kg). One of the other forms of terminal oxidases (e.g., P448) can be induced to a much higher extent without a dramatic increase in the “total” cytochrome P450.

The primary objective of the current study was to assess the potential role of metabolic activation (N-oxidation) in the acute toxicity of primary aromatic amines to fish. Activation has been established as a necessary factor in the chronic toxicity of these compounds in mammals (Bus and Popp, 1987; Beland and Kadlubar, 1985). In addition, N-oxidation of primary aromatic amines is seemingly due to P448 isoenzymes (Yamazoe et al., 1984). The data from Table 3, however, show that the acute LC50’s for aniline and 4-chloroaniline were not affected by induction. The LC50’s for both compounds for the induced and noninduced fish are essentially equal.

Assuming that the production of N-hydroxy metabolites increased with the observed induction, these results suggest that metabolic activation is not a factor in the
acute toxicity of these compounds. These results would suggest that the acute toxicity of these compounds may be adequately explained by assuming that they act under a narcosis mechanism distinct from baseline narcosis (Veith and Broderius, 1987; Bradbury et al., 1989).

Of course these results are not definitive. Although cytochrome P450 activity was induced in the Aroclor-treated fish (as measured by B[a]P hydroxylase activities), we do not have direct evidence that N-oxidation activity was increased. In addition, induction may also have increased rates of para or ortho ring hydroxylation, leading to increased detoxification, as well as activation, with the net effect being no change in LC50 values. Preliminary data, however, suggest that N-acetylation is the primary detoxification reaction in fish (Bradbury, unpublished data).

CONCLUSION

Although aromatic amine activation occurs in fish (Bradbury and Dady, unpublished data), the results of this study indicate that the role of activation in acute toxicity may be minimal. These results should not be extended to interpretations of the chronic toxicity of aromatic amines in fish. On the contrary, we expect that the formation of N-hydroxylated derivatives is critical for the erythrocyte toxicity and gas bladder tumorigenesis observed in chronic aniline- and 4-chloroaniline-exposed medaka (R. Johnson, USEPA, ERL-Duluth, unpublished data). More studies on the metabolism of aromatic amines in fish are needed to establish the role of N-oxidation in the acute and chronic toxicity of these compounds to fish.

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CYTOCHROME P450 INDUCTION: TOXICITY TO RAINBOW TROUT


