Metabolism of Chrysene by Brown Bullhead Liver Microsomes

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We have investigated the regio- and stereoselective metabolism of chrysene, a four-ring symmetrical carcinogenic polycyclic aromatic hydrocarbon (PAH), by the liver microsomes of brown bullhead (*Ameiurus nebulosus*), a bottom-dwelling fish species. The liver microsomes from untreated and 3-methylcholanthrene (3-MC)-treated brown bullheads metabolized chrysene at the rate of 30.1 and 82.2 pmol/mg protein/min, respectively. Benzo-ring diols (1,2-diol and 3,4-diol) were the major chrysene metabolites formed by liver microsomes from control and 3-MC-treated fish. However, the control microsomes produced a considerably higher proportion of chrysene 1,2-diol (benzo-ring diol with a bay region double bond) plus 1-hydroxychrysene, than 3,4-diol plus 3-hydroxychrysene, indicating that these microsomes are selective in attacking the 1,2- position of the benzene ring. On the other hand, 3-MC-induced microsomes did not show such a regioselectivity in the metabolism of chrysene. Control bullhead liver microsomes, compared to control rat liver microsomes, produced a considerably higher proportion of chrysene 1,2-diol, the putative proximate carcinogenic metabolite of chrysene. Like rat liver microsomes, bullhead liver microsomes produced only trace amounts of the K-region diol. Chrysene 1,2-diol and 3,4-diol formed by the liver microsomes from both control and 3-MC-treated bullheads consisted predominantly of their R,R-enantiomers. Chrysene is metabolized by bullhead liver microsomal enzymes to its benzo-ring diols with a relatively lower degree of stereoselectivity compared to benzo[a]pyrene (a five-ring PAH), but with a higher degree of stereoselectivity compared to phenanthrene (a three-ring PAH). The data of this study, together with those from our previous studies with phenanthrene, benzo[a]pyrene and dibenzo[a,l]pyrene (a six-ring PAH), indicate that the regioselectivity in the metabolism of PAHs in aquatic organisms in order to assess the carcinogenic potential of the hydrocarbons in these organisms.

The presence of these chemicals in the aquatic environment is of concern because of their mutagenic/carcinogenic properties. A high incidence of liver tumors has been reported in several species of bottom-dwelling fish collected from areas contaminated with these chemicals (Bauman *et al.*, 1987; Murchelano and Wolfe, 1985). It is well known that these chemicals require metabolic activation for exerting their carcinogenic effects (Conney, 1982). Therefore it is important to have information on the metabolism of PAHs in aquatic organisms in order to assess the carcinogenic potential of the hydrocarbons in these organisms.

In order to exert their carcinogenic effects, PAHs must first be bioactivated to bay-region diol epoxides via dihydrodiols with a bay-region double bond by the combined action of cytochrome P450-dependent monooxygenases and epoxide hydrolase (Conney, 1982). The cytochrome P450-dependent monooxygenase system oxygenates a PAH at several different sites. Therefore, the position of the molecule where the primary oxygenation occurs (regioselectivity) is an important determinant of the carcinogenic activity of PAH metabolites.

There is considerable evidence showing that teleost fish biotransform PAHs to metabolites that are similar to those reported for rodents (Egass and Varanasi, 1982; Pangrekar *et al.*, 1995, in preparation; Sikka *et al.*, 1990; Swain and Melius, 1984; Varanasi *et al.*, 1986; Yuan *et al.*, 1999). Our previous studies with benzo[a]pyrene (BaP; a five-ring PAH; Sikka *et al.*, 1990), dibenzo[a,l]pyrene (DB[a,1]P; a six-ring PAH; Yuan *et al.*, 1999), and phenanthrene (a three-ring PAH; Pangrekar *et al.*, 1995, in preparation) have shown that fish liver microsomes, in contrast to rat liver microsomes, metabolize the PAHs predominantly in the benzo-ring with much less oxidation at the K-region of the molecule, suggesting differences in the regioselectivity of the fish and rat liver cytochrome P450 system in the metabolism of these molecules. Our studies have also shown that among benzo-ring diols, brown bullhead (*Ameiurus nebulosus*) and trout liver microsomes produce a higher proportion of the dihydrodiols with a bay-region double bond (putative proximate carcinogenic metabolites) than they do of bay-region diols. The regioselectivity in the metabolism of PAHs by rat liver microsomes varies considerably with the size and shape of the molecule (Thakker *et al.*, 1985). In order to investigate whether molecular features influence the regio-

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**Key Words:** fish; brown bullhead; *Ameiurus nebulosus*; rat; chrysene; metabolism; liver microsomes; regioselectivity; stereoselectivity.

Polynuclear aromatic hydrocarbons (PAHs) constitute an important class of environmental contaminants (Harvey, 1991).

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selective metabolism of PAHs by fish, we extended our studies to the metabolism of chrysene (a four-ring symmetrical carcinogenic PAH that differs from BaP, DB[a,1]P, and phenanthrene with respect to molecular shape and size) by brown bullhead liver microsomes. Significant levels of chrysene have been found in sediments from the Great Lakes region (Bauman et al., 1987; Black, 1982) and from coastal regions in the U.S. (Gardner et al., 1991; Malins et al., 1985).

Stereochemical factors, including absolute and relative configuration of the diols and bay-region diol epoxides, also play a critical role in the expression of the carcinogenic effects of these compounds, because among various possible stereoisomers, only R,R-diols and R,S-diol–S,R-epoxides exhibit exceptionally high carcinogenic activity (Thakker et al., 1985). Our earlier studies showed that brown bullhead liver microsomes metabolize BaP to 7R,8R-diol with a high degree (90%) of stereoselectivity (Sikka et al., 1990), while phenanthrene is metabolized to 1R,2R-diol with less than 60% stereoselectivity. Our earlier studies showed that brown bullhead liver microsomes metabolize BaP to 7R,8R-diol with a high degree (90%) of stereoselectivity (Thakker et al., 1985). We have compared our data with those reported for the metabolism of chrysene by rats (Nordqvist et al., 1981). We have reported part of this work in abstract form (Pangrekar et al., 1995).

MATERIALS AND METHODS

Chemicals. [G-3H]-chrysene (specific activity 1.42 Ci/mmol) was obtained from the NCI Radiochemical Reference Standard Repository. It was purified to 98.7% by HPLC using a Zorbax ODS column (0.65 × 25 cm) run isocratically with a solvent of water:methanol (40:60; Nordqvist et al., 1981). Chrysene and its synthetic metabolite standards chrysene 1,2-, 3,4-, and 5,6-diol, chrysene 5,6-quinone and 1,2-,3,4- and 6-hydroxychrysene were obtained from the NCI Chemical Carcinogen Reference Standards Repository. (-)-Chrysene 1,2,3,4-tetrahydro-1,2-diol was synthesized according to the published procedure (Karle et al., 1977). NADPH was purchased from Sigma Chemical Co. (St. Louis, MO).

Treatment of fish and preparation of liver microsomes. Brown bullheads (125–175 g) were obtained from the Zetts Fish Hatchery (Drifting, PA). The fish were held in flowing charcoal-filtered dechlorinated city water at a temperature of 18–20°C, under a 12:12 h light-dark photoperiod. They were acclimated for a period of 4 weeks and fed laboratory fish chow daily (Ziegler Brothers, Inc., Gardener, PA). The fish were injected (ip) with 3-methylcholanthrene (3-MC) at a dose of 20 mg/kg in corn oil (Pangrekar et al., 1995); control fish received an equal amount of corn oil. The fish were fed ad libitum and maintained in flowing water during the induction period. Groups of six to eight fish were sacrificed by severing the spinal cord five days after treatment.

The livers were rapidly excised into ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and maintained in flowing water during the induction period. Groups of six to eight fish were sacrificed by severing the spinal cord five days after treatment. The livers were rapidly excised into ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl, gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted. The pooled livers were minced, and immediately homogenized into 4 volumes of ice-cold 0.1 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl and gently blotted and weighted.

Metabolism of chrysene by liver microsomes. The liver microosomal incubation mixture (total volume 1.0 ml) containing 80 µmol of potassium phosphate buffer (pH 7.4), 3 µmol of MgCl2, 1.1 µmol of NADPH, and 0.5 mg of microsomal protein from control or 3-MC-induced liver microsomes was preincubated for 3 min at 25°C. The reaction was initiated thereafter by the addition of 15 or 5 µM of [1H]chrysene (approximately 2.0 µCi in 20 µl of DMSO) for control and 3-MC-induced microsomes, respectively. Incubation mixtures without NADPH served as controls. The reaction was carried out in a shaking water bath for 20 min. The extent of chrysene metabolism was determined according to the procedure of Van Cantfort et al. (1977).

For analysis of chrysene metabolites, the reaction was terminated by the addition of 1 ml of ice-cold acetone after 20 min of incubation. The incubation mixture was extracted three times with two volumes of ethyl acetate. The ethyl acetate layers containing chrysene and its metabolites were pooled, dried over anhydrous sodium sulfate, concentrated, and the residue was stored at –80°C until HPLC analysis. All experiments were conducted under low UV yellow light to minimize photodegradation of the chemicals.

HPLC analysis of chrysene metabolites. Prior to HPLC analysis, concentrated extracts of incubated samples were dissolved in 0.1 ml of freshly distilled tetrahydrofuran (THF). An aliquot of the extract was mixed with appropriate synthetic reference standards (chrysene 1,2-, 3,4-, and 5,6-diol, 1-,2-, 3,4-, and 6-hydroxychrysene, and chrysene 5,6-quinone) and chrysene and its metabolites were resolved on a Varian 5000 HPLC equipped with a Zorbax ODS column (5 µm, 25 cm × 4.0 mm, id), a solvent programmer, and a variable wavelength uv/visible detector, set at 280 nm. The column was eluted with a nonlinear gradient of 60–100% methanol in water over a period of 40 min after an initial delay of 5 min, at a flow rate of 1.2 ml/min (Nordqvist et al., 1987).
et al., 1981). Eluent from the HPLC column was collected every 20 s directly into scintillation vials using an ISCO-Foxy fraction collector, scintillation fluid (Scintiverse E, Fisher Scientific) was added to the fractions and the radioactivity was determined using a Beckman LS 3801 liquid scintillation counter. Chrysene metabolites were identified by comparing their retention times with those of authentic standards. The radiolabeled metabolites were quantitated by summing the radioactivity in fractions corresponding in retention time and peak width to peaks of authentic standards. In calculating the metabolism of chrysene, appropriate corrections were made for the values obtained with blanks. The overall recovery of radioactivity from the HPLC column was >95%.

We noted that 1- and 4-hydroxychrysene and 2-and 3-hydroxychrysene, the two pairs of chrysene-derived phenols, coeluted in the solvent system described above. To separate 1- and 4-hydroxychrysene, the column was eluted isocratically with 62% methanol in water containing 1% N-butylamine (Tjessum and Stegeman, 1979). Because a relatively low amount of radioactivity coeluted with 2- and 3-hydroxychrysene, no further attempt was made to resolve the two phenols.

**Resolution of enantiomers of benzo ring diols of chrysene.** Metabolically formed [3H]chrysene 1,2- and 3,4-diols were isolated by reverse phase HPLC as described above and were evaporated to dryness under N2. The 3,4-diol was converted to tetrahydrodiol by dissolving it into 3 ml of THF and bubbling with hydrogen gas for 30 min in the presence of Adams Catalyst (Piò, Weems et al., 1986). The 1,2-diol and 3,4-tetrahydrodiol were mixed with UV detectable amounts of the corresponding synthetic racemic standards. The enantiomers were resolved by normal phase HPLC on a chiral column (Pirkle 1-A, 4.6 mm id x 25 cm., Regis Chemical Company, Morton Grove, IL) packed with (R)-N-(3,5-dinitrobenzoyl)phenylglycine ionically bonded to y-amino propyl silicic silica (Weems et al., 1986). The column was eluted with hexane:ethanol:acetoneitrile (27:2:1 v/v) at a flow rate of 1.2 ml/min at room temperature. The eluate was monitored at 280 nm. The R,R and S,S peaks were designated on the basis of their elution order reported in the literature (Weems et al., 1986). The peaks corresponding with the R,R and S,S enantiomers were collected and counted for radioactivity.

**RESULTS**

**Rate of Metabolism of Chrysene by Bullhead Liver Microsomes**

Initial experiments were performed to establish linear conditions for the metabolism of chrysene by liver microsomes from control and 3-MC treated bullheads, with respect to substrate concentration, incubation time, and microsomal protein concentration. Chrysene metabolism was measured according to Van Cantfort et al. (1977) using 15 μM of chrysene for 20 min for microsomal protein linearity, 15 μM of chrysene and 1 mg of microsomal protein/ml for time linearity, and 1 mg microsomal protein/ml for 20 min for substrate linearity. The reaction was linear to 1 mg and 0.5 mg of protein/ml for control and 3-MC-induced microsomes, respectively. With microsomes from control fish, chrysene metabolism was linear up to substrate concentration of 15 μM. However, with liver microsomes from 3-MC-treated fish, the reaction was linear only up to 5 μM substrate concentration. The time course of metabolism was linear for 25 min for both control and 3-MC-induced microsomes.

The rate of metabolism of chrysene by liver microsomes and the profile of metabolites formed were examined at a saturating substrate concentration (15 and 5 μM for control and 3-MC-induced microsomes, respectively) under conditions that gave linearity with respect to microsomal protein concentration (0.5 mg/ml) and incubation time (20 min). The metabolism of chrysene by control microsomes was 30.1 ± 2.53 pmol/min/mg of microsomal protein. The rate was nearly 2.7-fold higher (82.2 ± 0.71 pmol/min/mg protein) for liver microsomes from 3-MC-pretreated fish. These values represent ± SD of triplicate determinations.

**Profile of Chrysene Metabolites Formed by Bullhead Liver Microsomes**

A typical profile of ethyl acetate-soluble chrysene metabolites formed by bullhead liver microsomes incubated with [3H] chrysene for 20 min is shown in Figure 2. The following chrysene metabolites were identified on the basis of co-chromatography with authentic standards: chrysene 1,2-, 3,4-, and 5,6-dihydrodiol, 1- and 4-hydroxychrysene, and 2-/3-hydroxychrysene. Table 1 shows the relative proportion of the metabolites formed by control and 3-MC-induced microsomes. Benzo-ring diols (1,2-diol and 3,4-diol) represented the major chrysene metabolites formed by control liver microsomes. Chrysene 1,2-diol with bay region double bond (putative proximate carcinogenic metabolite) and chrysene 3,4-diol accounted for 58 and 24%, respectively of the total ethyl acetate-extractable metabolites formed by control microsomes. The K-region diol (5,6-diol) was formed in only trace amounts (∼1%). The phenol fraction (1-, 2-3-, 4-, and 6-hydroxychrysene) constituted approximately 13% of total chrysene metabolites.

The microsomes from 3-MC-treated fish, like control microsomes, produced benzo-ring diols as the major chrysene metabolites. However, the relative proportions of the two benzo-ring diols formed by the two types of microsomes were
considerably different. The 3-MC-induced microsomes produced a lower proportion of chrysene 1,2-diol (16.1%) compared to chrysene 3,4-diol (28.1%). The reverse was observed for control microsomes, i.e., the percentage of 1,2-diol formed was substantially higher than that of 3,4-diol. Two other major metabolites formed by 3-MC-induced microsomes were 1-hydroxchrysene (17%) and 4-hydroxchrysene (8.5%). In addition, trace amounts of chrysene 5,6-diol, 5,6-quinone, 2-/3- and 6-hydroxychrysene were formed.

### Stereoselectivity in the Metabolic Formation of Chrysene Diols

Enantiomeric composition of chrysene 1,2-diol and chrysene 3,4-diol formed by liver microsomes of control and 3-MC-treated brown bullheads was determined using a chiral column (Weems et al., 1986). Since (±)-chrysene 3,4-diol, unlike its hydrogenated product (±)-chrysene 1,2,3,4-tetrahydro-3,4-diol, can not be resolved into its (+)- and (−)-enantiomers on the chiral column (Weems et al., 1986), the metabolically formed chrysene 3,4-diol was catalytically hydrogenated to chrysene 1,2,3,4-tetrahydro-3,4-diol prior to chromatographic separation. The percentage of each enantiomer of the metabolically formed chrysene 1,2-diol and chrysene 3,4-diol was determined from the amount of radioactivity coeluting with the corresponding R,R and S,S enantiomers of the authentic diols (Figs. 3 and 4). Chrysene 1,2-diol formed by both control and 3-MC-induced liver microsomes contained the R,R enantiomer to the extent of 70–80% (Table 2). However, the enantiomeric purity of the R,R-enantiomer of metabolically formed chrysene 3,4-diol was >94%. Because of the trace amount of metabolically formed chrysene 5,6-diol, no attempt was made to establish its enantiomeric composition.

### DISCUSSION

PAHs require metabolic activation by the cytochrome P450-dependent monooxygenase system in order to express their mutagenic/carcinogenic effects (Conney, 1982). Both the regio- and stereoselectivity of the enzymes involved in the biotransformation of PAHs play a major role in the carcinogenicity of these chemicals (Thakker et al., 1985). We have previously reported data on the regio- and stereoselective metabolism of BaP (Sikka et al., 1990) and phenanthrene (Pangrekar et al., 1995, in preparation) by brown bullhead liver microsomes, and on the regioselective metabolism of DB[a,l]P by trout liver.

### TABLE 1

Profile Of [3H] Chrysene Metabolites Formed by Brown Bullhead and Rat Liver Microsomes

<table>
<thead>
<tr>
<th>Chrysene metabolites</th>
<th>Bullhead liver microsomes</th>
<th>Rat liver microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 3-MC-induced</td>
<td>Control 3-MC-induced</td>
</tr>
<tr>
<td>1,2-Diol</td>
<td>57.6 ± 2.5 (17.33 ± 0.75)</td>
<td>61.6 ± 0.9 (4.85 ± 0.27)</td>
</tr>
<tr>
<td>3,4-Diol</td>
<td>23.8 ± 1.4 (7.16 ± 0.42)</td>
<td>28.1 ± 2.5 (8.46 ± 0.75)</td>
</tr>
<tr>
<td>5,6-Diol</td>
<td>1.0 ± 0.4 (0.30 ± 0.12)</td>
<td>3.1 ± 0.7 (0.93 ± 0.21)</td>
</tr>
<tr>
<td>5,6-Quinone</td>
<td>2.0 ± 0.0 (0.8 ± 0)</td>
<td>2.0 ± 0.0 (0.8 ± 0)</td>
</tr>
<tr>
<td>1-HO-CHR</td>
<td>3.9 ± 0.3 (1.17 ± 0.09)</td>
<td>17.2 ± 1.2 (5.18 ± 0.36)</td>
</tr>
<tr>
<td>2,3-HO-CHR</td>
<td>7.5 ± 0.6 (2.25 ± 0.18)</td>
<td>2.8 ± 0.2 (0.84 ± 0.06)</td>
</tr>
<tr>
<td>4-HO-CHR</td>
<td>1.5 ± 0.1 (0.45 ± 0.03)</td>
<td>8.5 ± 0.6 (2.56 ± 0.18)</td>
</tr>
<tr>
<td>6-HO-CHR</td>
<td>0.7 ± 0.0 (0.21 ± 0)</td>
<td>0.2 ± 0.0 (0.06 ± 0)</td>
</tr>
<tr>
<td>Unknown metabolites</td>
<td>4.7</td>
<td>22.0</td>
</tr>
</tbody>
</table>

**Note.** Values are % of total metabolites.

*Values obtained by incubating 15 mM chrysene (control microsomes) or 5 mM chrysene (3-MC-induced microsomes) for 20 min at a microsomal protein concentration of 0.5 mg/ml. Values are mean ± SD of triplicate determinations and represent the percentage of total radioactivity that emerges from HPLC column in the defined metabolite fractions prior to chrysene. The figures in parentheses represent the amount of chrysene metabolites formed (pmol/min/mg microsomal protein).

*Data taken from Nordqvist et al. (1981).
microsomes (Yuan et al., 1999). Since the size and shape of a PAH influence the regio- and stereoselectivity in the metabolism of PAHs (Thakker et al., 1985), we have extended our studies to the metabolism of chrysene, a four-ring symmetrical PAH, by brown bullhead liver microsomes in order to obtain a better understanding of the metabolism of PAHs by fish. We have compared our data with those reported for the metabolism of chrysene by rat liver microsomes (Nordqvist et al., 1981).

A comparison of the rate of metabolism of chrysene with that of BaP and phenanthrene by liver microsomes from control and 3-MC-treated bullheads indicates that the substrate specificity of the bullhead liver microsomal enzymes for the three PAHs varies considerably. The control and 3-MC-induced microsomes metabolized chrysene at approximately 0.78 to 0.13 the rate of metabolism of BaP, and 2 to 4 times the rate of metabolism of phenanthrene, respectively (Pangrekar et al., 1995, in preparation). Compared to rat liver microsomes (Nordqvist et al., 1981), the liver microsomes from control and 3-MC-treated bullheads metabolized chrysene at a considerably lower rate. Furthermore, the liver microsomes from bullhead and rat differ considerably from each other with respect to their relative substrate specificity for chrysene, BaP, and phenanthrene. Compared to phenanthrene, chrysene was metabolized at a higher rate by bullhead liver microsomes (Pangrekar et al., 1995, in preparation) but at a lower rate by rat liver microsomes (Nordqvist et al., 1981).

The regiospecific metabolism of chrysene by bullhead liver microsomes is similar to that of two other PAHs, BaP (Sikka et al., 1990) and phenanthrene (Pangrekar et al., 1995, in preparation). All three hydrocarbons are converted to their K-region diols to a minor extent but are metabolized substantially to benzo-ring diols, which account for as much as 81% of the total metabolites in the case of chrysene. Like BaP and phenanthrene, chrysene is converted to a diol with a bay-region double bond (1,2-diol; proximate carcinogenic metabolite) to a much greater extent than to a bay-region diol (3,4-diol) by control bullhead liver microsomes. The diol with a bay-region double bond represented as much as 57.6% of the total chrysene metabolites compared to 14.8 and 25.3% in the case of BaP and phenanthrene, respectively. These data indicate that among the three PAHs, chrysene is converted to a precursor of a bay-region diol epoxide (the ultimate carcinogenic metabolite) to the greatest extent by bullhead liver microsomes.

Although the types of chrysene metabolites formed by control and 3-MC-induced liver microsomes were similar, there were considerable differences in the relative proportions of the individual metabolites formed by the two types of microsomes. A striking feature of chrysene metabolism by control microsomes was that, in comparison to 3-MC-induced microsomes, the control microsomes produced 3.5 times greater percentage of 1,2-diol. The control microsomes produced a much higher proportion of 1,2-diol plus 1-hydroxychrysene than of 3,4-diol plus 3-hydroxychrysene, indicating that these microsomes are selective in their attack at the 1,2-position of the benzo ring. In contrast, 3-MC-induced microsomes did not exhibit such a regioselectivity since the proportion of chrysene metabolites formed via oxidation at the 1,2-double bond (16.1% of 1,2-diol plus 17.2% of 1-hydroxychrysene) was nearly identical to that of the metabolites formed via oxidation at the 3,4-double bond (28.1% of chrysene 3,4-diol plus 8.5% of 4-hydroxychrysene). These data suggest that the cytochrome(s) P450 in uninduced

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**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1,2-Diol</th>
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<th>3,4-Diol</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>(−) [1R,2R]</td>
<td>(+) [1S,2S]</td>
<td>Enantiomeric purity (%)</td>
<td>(−) [3R,4R]</td>
</tr>
<tr>
<td>Control</td>
<td>89% ± 0.38</td>
<td>11% ± 0.71</td>
<td>78</td>
<td>97%</td>
</tr>
<tr>
<td>3-MC</td>
<td>85% ± 1.41</td>
<td>15% ± 2.12</td>
<td>70</td>
<td>97%</td>
</tr>
</tbody>
</table>

*Note.* The data represent the mean ± SD of triplicate determinations. Percent enantiomeric purity is defined as 100 (mol predominant enantiomer R,R-mol S,S)/(mol R,R + mol S,S; Kumar et al., 1995).
microsomes is more efficient in oxidizing the chrysene 1,2-double bond than the cytochrome(s) P450 in 3-MC-induced microsomes.

A comparison of the profile of chrysene metabolites formed by bullhead liver microsomes (this study) and rat liver microsomes (Nordqvist et al., 1981) showed that the corresponding microsomes from the two species differ considerably with respect to the regioselective metabolism of the hydrocarbon. Compared to control rat liver microsomes, control bullhead liver microsomes produced a considerably greater proportion of chrysene 1,2-diol, the putative proximate carcinogenic metabolite of chrysene. Unlike control bullhead liver microsomes, control rat liver microsomes do not exhibit any regioselectivity in the oxidative attack at the 1,2- and 3,4- positions of the benzo ring. On the other hand, 3-MC-induced rat liver microsomes were more efficient at oxidizing the 3,4-double bond than the 1,2-double bond.

The liver microsomes from control and 3-MC-treated bullheads showed a high degree of stereoselectivity in the metabolism of chrysene to 1,2-diol and 3,4-diol, with the (–)–(R,R) enantiomer predominating in each case. However, the enantioselective purity was somewhat less for chrysene 1,2-diol (70–78%) than that observed for chrysene 3,4-diol (94%). These data are comparable to those reported for the liver microsomes from 3-MC-treated rats that metabolized chrysene to 1R, 2R- and 3R, 4R-diol with 80–97% enantioselective purity (Nordqvist et al., 1981). However, unlike control bullhead liver microsomes, which show a high degree of enantiomeric selectivity in the formation of chrysene 1,2-diol, control rat liver microsomes showed far less enantiomeric specificity in the formation of 1,2-diol. It appears that, in contrast to what was noted with rats, 3-MC treatment of bullheads does not alter the stereoselectivity of the enzymes (cytochrome P450 and epoxide hydrolase) responsible for metabolizing chrysene to its diols.

A comparison of these studies with our earlier studies on the stereoselective metabolism of BaP (Sikka et al., 1990) and phenanthrene by bullhead liver microsomes (Pangrekar et al., 1995, in preparation) shows that chrysene is metabolized to its benzo-ring diol having a bay-region double bond with a lower degree of stereoselectivity than BaP. However, the degree of stereoselectivity in the metabolism of chrysene to its benzo-ring diols is considerably higher than that noted with phenanthrene. The data indicate that the degree of stereoselectivity in the metabolism of PAHs by bullhead liver microsomes varies with the size and shape of the molecule and follows the order: BaP > chrysene > phenanthrene. These findings suggest that chrysene and phenanthrene, unlike BaP, are metabolized by more than one cytochrome P450 isozyme, presumably with different stereoselectivities. The observed stereoselectivity of fish liver microsomal enzymes in the metabolism of PAHs is of toxicological significance because PAH diols with an [R,R] configuration are considerably more carcinogenic than diols with an [S,S] configuration (Thakker et al., 1985).

In summary, the results of this study, in conjunction with our previous studies with BaP (Sikka et al., 1990), DB[a]IP (Yuan et al., 1999), and phenanthrene (Pangrekar et al., 1995, in preparation), show that chrysene, like the other two PAHs, is metabolized predominantly to benzo-ring diols by bullhead liver microsomes. These data appear to indicate that regioselectivity in the metabolism of PAHs by fish liver microsomes does not vary greatly with the size and shape of the molecule, whereas the degree of stereoselectivity in the metabolism of PAHs to benzo-ring diols does. Our data on the regioselective metabolism of chrysene, particularly with regard to the metabolic attack at the benzo-ring, and the stereoselective metabolism of the hydrocarbon, are different from what has been reported for rat liver microsomes (Nordqvist et al., 1981). These differences may be due to variations in the relative amounts of various cytochrome P450 and epoxide hydrolase isozymes involved in the metabolism of the hydrocarbon by the hepatic microsomes of brown bullhead and rats.

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