HYDRATION OF ARENE OXIDES OF POLYCYCLIC HYDROCARBONS

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The stereospecific metabolism of racemic benzo[a]pyrene 4,5-, 7,8-, and 9,10-oxide by rat liver microsomes or highly purified epoxide hydrase has been examined. The enantiomeric purity of the metabolically formed benzo[a]pyrene 7,8- and 9,10-dihydrodiol is relatively low (8% and 22%, respectively) whereas metabolically formed benzo[a]pyrene 4,5-dihydrodiol appears to be highly enriched in the (−)-enantiomer (78% enantiomeric purity). The low optical purity of benzo[a]pyrene 7,8-dihydrodiol appears to be due to the ability of epoxide hydrase to act upon position 8 of both optical enantiomers of benzo[a]pyrene 7,8-oxide with almost equal ease. Metabolism of racemic benzo[a]pyrene 7,8-oxide in 100% enriched water indicated that this substrate was hydrated almost exclusively (>98%) at position 8 by the membrane-bound or highly purified enzyme, whereas racemic benzo[a]pyrene 4,5-oxide was attacked by water with almost equal ease at positions 4 and 5 of the molecule.

Other polycyclic hydrocarbon K region dihydrodiols, such as phenanthrene 9,10-dihydrodiol and benz[a]anthracene 5,6-dihydrodiol, are formed with less stereospecificity (28% and 42% optical purity, respectively) than benzo[a]pyrene 4,5-dihydrodiol. The above observations indicate that the degree of stereospecific hydration of arene oxides by epoxide hydrase depends on the substrate studied. The type and extent of specificity appears to be governed by both steric and electronic factors associated with the substrate itself.

The liver microsomal cytochrome P-450-containing monooxygenase system and epoxide hydrase play important roles in determining the biological fate of many environmental pollutants and toxic compounds. For many of these chemicals such as the polycyclic aromatic hydrocarbons, biologically active arene oxides are the initial products formed by the microsomal cytochrome P-450 system (1, 2). Certain of these arene oxides have toxic, mutagenic, and carcinogenic activity (1–6). The microsomal enzyme epoxide hydrase plays a pivotal role in the metabolism of these intermediate arene oxides to dihydrodiols via the trans addition of water (1). The dihydrodiols formed are usually biologically inactive per se, but some of these compounds are further metabolized by the cytochrome P-450-dependent monooxygenase system to highly mutagenic and carcinogenic diol epoxides (7–10) which are poor substrates for epoxide hydrase (7, 11–13). Thus, epoxide hydrase plays a central role in both the inactivation and activation of polycyclic aromatic hydrocarbons to mutagenic and carcinogenic metabolites.

Both the cytochrome P-450 system and epoxide hydrase show varying and often very high degrees of stereospecificity in their metabolism of different substrates. For example, liver microsomes from 3-methylcholanthrene-treated rats oxidize the (−)-enantiomer of BP 7,8-dihydrodiol primarily (86%) to BP 7,8-diol-9,10-epoxide with the i-OH group and the oxirane oxygen having trans stereochemistry (14, 15), whereas under similar conditions the (+)-enantiomer is almost exclusively (97%) oxidized to BP 7,8-diol-9,10-epoxide in which the 7-OH group and oxirane oxygen have cis stereochemistry (14, 15). The 4,5-, 7,8-, and 9,10-dihydrodiols formed metabolically from benzo[a]pyrene were found to have high optical purity (14). However, BP 7,8-dihydrodiol formed from the racemic BP 7,8-oxide was of very low optical purity (14) indicating low stereospecificity in their metabolism of different substrates. For example, liver microsomes from 3-methylcholanthrene-treated rats oxidize the (−)-enantiomer of BP 7,8-dihydrodiol primarily (86%) to BP 7,8-diol-9,10-epoxide with the i-OH group and the oxirane oxygen having trans stereochemistry (14, 15), whereas under similar conditions the (+)-enantiomer is almost exclusively (97%) oxidized to BP 7,8-diol-9,10-epoxide in which the 7-OH group and oxirane oxygen have cis stereochemistry (14, 15). The 4,5-, 7,8-, and 9,10-dihydrodiols formed metabolically from benzo[a]pyrene were found to have high optical purity (14). However, BP 7,8-dihydrodiol formed from the racemic BP 7,8-oxide was of very low optical purity (14) indicating low stereospecificity in their metabolism of different substrates.
specificity of rat liver epoxide hydrase for this substrate. Since the BP 7,8-dihydrodiol formed from benz[a]pyrene by rat liver is of high optical purity, the cytochrome P-450-dependent monoxygenase system must form BP 7,8-oxide with high optical purity. Epoxide hydrase from rabbit liver is highly stereospecific toward the 9,10-oxide of phenanthrene, forming 9,10-dihydrodiol with high optical purity (60 to 70%) but less stereospecific toward benzene oxide (optical purity of the dihydrodiol ~30-50%) (17). Epoxide hydrase from rabbit liver catalyzes the hydration of cis stilbene oxide to give the corresponding dihydrodiol of high optical purity (18). Since the stereospecific metabolism of chemicals by the cytochrome P-450 system and epoxide hydrase can play a critical role in the expression of the biological activity of these chemicals, we have studied the stereochemical course of hydration of several carcinogenic and noncarcinogenic arene oxides.

MATERIALS AND METHODS

Chemicals—Racemic [3-^H]phenanthrene 9,10-oxide (1.23 μCi/μmol), [7-^H]benz[a]anthracene 5,6-oxide (9.05 μCi/μmol), [6-^H]BP 4,5-oxide (10.0 μCi/μmol), [6-^H]BP 7,8-oxide (10.9 μCi/μmol), and [6-^H]BP 9,10-oxide (8.4 μCi/μmol) were synthesized as previously described (19). The dihydrodiols formed enzymatically from these substrates are shown in Fig. 1. Stock solutions of the substrates were prepared in tetrahydrofuran containing 0.1% ammonia and stored at -90° prior to use. (-)-α-Trifluoromethylphenylacetic acid (Aldrich) was refluxed with thionyl chloride in pyridine for 50 h to obtain the corresponding acid chloride (MTPA-Cl). In most cases, incubation conditions were selected to limit the conversion of the arene oxides to dihydrodiols to less than 20% of the added substrate.

Enzyme Preparations—Immature (50 to 60 g) male rats of the Long-Evans strain were treated with phenobarbital (75 mg/kg per day) or 3-methylcholanthrene (25 mg/kg per day in corn oil) for 4 days. Microsomes were prepared as previously described (20) and stored at -90°. Epoxide hydrase was purified to apparent homogeneity as described (21).

Hydration of Polycyclic Hydrocarbon Arene Oxides—The standard incubation mixture consisted of 0.12 μmol of Tris/HCl buffer (pH 9.0 at 23°), microsomes (2 to 4 mg of protein), tritiated substrate (40 pmol), and water to reach a final volume of 1.00 ml. Reactions were initiated by addition of the substrate and the reaction mixture was incubated at 37° for 2 to 10 min after which 3.0 ml of ethyl acetate was added to the incubation mixture and the product as well as the unreacted arene oxide were extracted into the organic phase. The ethyl acetate layer was separated by centrifugation and dried (anhydrous Na₂SO₄), and the solvent was evaporated with a stream of dry nitrogen. The residue was dissolved in a small volume of tetrahydrofuran (~50 μl) and the dihydrodiols were isolated by HPLC. With purified epoxide hydrase, the experimental conditions were similar except that highly purified epoxide hydrase (30 μg) and dilauroyl phosphatidylcholine (600 μg) were used instead of microsomes.

Optical Purity of Dihydrodiols—[^H]-labeled dihydrodiols obtained from the reactions of the corresponding arene oxides with epoxide hydrase were diluted with a large excess (~1 mg) of the same unlabeled, racemic dihydrodiol as carrier and were dissolved in 100 μl of pyridine. An excess of MTPA-Cl was then added and the reaction mixture was allowed to stand under N₂ at 4° for 12-15 h. The pairs of diastereomeric diesters which formed were separated by HPLC and the radioactivity associated with each diastereomer was determined. Since the recovery of each member of the pair of diastereomers for each dihydrodiol was identical as determined by peak area, the enantiomeric purity of the enzymatically formed[^H]-labeled dihydrodiol could be calculated directly from the radioactivity in each peak. The concept is illustrated below:

\[
\begin{align*}
(-)^{-}\text{-MTPA-}- & (\text{+}-)\text{-dihydrodiol} \quad \text{Separation of diastereomers} \quad \text{and} \\
(\text{--})\text{-MTPA-}(\text{--})\text{-dihydrodiol} \quad \text{and quantitation} \\
\text{Diastereomeric mixture} \\
\end{align*}
\]

High Pressure Liquid Chromatography—HPLC analyses were performed with a Spectra-Physics model 3500 chromatograph or with a Du Pont model 841 chromatograph equipped with a Du Pont 838 programmable gradient. BP 4,5-, 7,8- and 9,10-dihydrodiols obtained from incubations of the corresponding acid chloride (MTPA-Cl) In most cases, incubation conditions were selected to limit the conversion of the arene oxides to dihydrodiols to less than 20% of the added substrate.

Enzyme Preparations—Immature (50 to 60 g) male rats of the Long-Evans strain were treated with phenobarbital (75 mg/kg per day) or 3-methylcholanthrene (25 mg/kg per day in corn oil) for 4 days. Microsomes were prepared as previously described (20) and stored at -90°. Epoxide hydrase was purified to apparent homogeneity as described (21).

Fig. 1. Dihydrodiols of phenanthrene, benz[a]anthracene, and benz[a]pyrene formed by epoxide hydrase from the corresponding arene oxides. Absolute stereochemistry is not implied.
was achieved on two such Zorbax SIL columns coupled in series. The elution conditions and the retention times of all the dihydrodiols and their bis MTPA diesters are given in Table I. Per cent conversion of the substrate in each case was computed by quantitating the total radioactivity emerging from the column. In all cases, arene oxides and their phenolic isomerization products were well separated from the dihydrodiols.

**RESULTS**

The enantiomeric purity of benzo[a]pyrene dihydrodiols, obtained by hydration of the corresponding racemic arene oxides with liver microsomal epoxide hydrolase, are given in Table II. A typical HPLC trace of the separation of the diastereomers from which the optical purity of the corresponding dihydrodiol was computed is shown in Fig. 2. The enantiomeric purity of the metabolically formed BP 7,8- and 9,10-dihydrodiols is relatively low (8% and 22%, respectively) whereas BP 4,5-dihydrodiol appears to be highly enriched in the (+)-enantiomer (78% enantiomeric purity). The low enantiomeric purity of the BP 7,8- and 9,10-dihydrodiols could be due to any of several reasons. For example, the enzyme epoxide hydrolase may lack specificity for positions 7 and 8 of the molecule or it may have comparable preference for both enantiomeric forms of the substrates. These possibilities were investigated when racemic BP 7,8-oxide was enzymatically hydrated in 18O-enriched water. The amount of incorporation of 18O into positions 7 and 8 of the dihydrodiol was determined by acid-catalyzed dehydration of the dihydrodiol to 7-HOBP which was then isolated by HPLC (14). None of the isomeric 8-HOBP was detected in the sample. BP 7,8-dihydrodiol, obtained from incubation in 18O-enriched water, showed 19% incorporation of 18O; however, 7-HOBP formed by acid-catalyzed dehydration of the dihydrodiol contained none of the 18O that was incorporated into the dihydrodiol. Therefore, hydration of racemic BP 7,8-oxide occurred exclusively (>98%) at position 8. As shown in Fig. 3, attack of water at position 8 of both enantiomers of BP 7,8-oxide at comparable rates to cause trans opening of the

**Table I**

| Chromatographic conditions for isolation of benzo[a]pyrene, benz[a]anthracene, and phenanthrene dihydrodiols and separation of their diastereomeric bis MTPA esters |
|-----------------------------------------------|
| Gradient conditions for: BP 4,5-dihydrodiol, 80 to 99% methanol/H2O in 10 min; BP 7,8-dihydrodiol, 85 to 99% methanol/H2O in 14 min; BP 9,10-dihydrodiol, 70 to 99% methanol/H2O in 29 min; BP 4,5-bis MTPA, 80 to 99% methanol/H2O in 20 min; BP 7,8-bis MTPA, 90 to 99% methanol/H2O in 9 min; benz[a]anthracene 5,6-bis MTPA, 90% methanol/H2O (isocratic). Benzo[a]pyrene dihydrodiols were monitored at 320 nm, BP 4,5-bis MTPA was monitored at 320 nm, BP 7,8-bis MTPA at 397 nm, and benz[a]anthracene 5,6-bis MTPA at 310 nm. |

| Dihydrodiol | Retention time of dihydrodiol (min) | Retention time of bis MTPA esters (min) |
|-------------|----------------------------------|----------------------------------------|
| BP 4,5-dihydrodiol | 10.5 | 90.0 |
| BP 7,8-dihydrodiol | 9.0 | 13.8 |
| BP 9,10-dihydrodiol | 10.5 | 66.0 |
| Benz[a]anthracene 5,6-dihydrodiol | 13.0 | 46.3 |
| Phenanthrene 9,10-dihydrodiol | 12.0 | 33.4 |

Zorbax ODS columns were used (6.2 mm × 25 cm) with methanol/H2O gradients at a flow rate of 1.2 ml/min. The gradient was started 1 min after injection of the sample.

Zorbax SIL columns were used (6.2 mm × 25 cm) with 3% tetrahydrofuran in cyclohexane as eluent at a flow rate of 3.1 ml/min. The products were monitored at 254 nm.

Zorbax SIL columns were used (6.2 mm × 25 cm) with 3% isopropyl alcohol and 10% dioxane in hexane as eluent at a flow rate of 5.0 ml/min. The products were monitored at 254 nm.

Two coupled Zorbax SIL columns (6.2 mm × 25 cm) were used with 0.15% isopropyl alcohol and 0.5% dioxane in hexane as eluent at a flow rate of 3.0 ml/min. The products were monitored at 254 nm.

**Table II**

| Enantiomeric purity of dihydrodiols formed during hydration of arene oxides of benzo[a]pyrene by liver microsomes from phenobarbital-treated rats |
|-----------------------------------------------|
| Racemic BP 4,5-, 7,8-, and 9,10-oxide were incubated with liver microsomes from phenobarbital-pretreated rats and the formation of (+) and (-)-enantiomers was quantified as described under "Materials and Methods." |

| Dihydrodiol formed | Benzo[a]pyrene arene oxide as substrate | % each enantiomer** | % enantiomeric purity** |
|---------------------|--------------------------------------|-------------------|-------------------------|
| BP 4,5-dihydrodiol  | 69 | 11 | 76 | 92 |
| BP 7,8-dihydrodiol  | 54 | 46 | 8 | 92 |
| BP 9,10-dihydrodiol | 61 | 39 | 22 | 92 |

* The sign of the enantiomers represents the sign of the CD curve at the longest wavelength transition which is 271 nm for BP 4,5-dihydrodiol, 397 nm for BP 7,8-dihydrodiol, and 298 nm for BP 9,10-dihydrodiol. Values of (+) and (-) are based on radioactivity associated with the separated diastereomeric MTPA diesters as described under "Materials and Methods."

** Per cent enantiomeric purity is defined as 100 (moles of l-form - moles of d-form) divided by (moles of l-form + moles of d-form). This value is identical to the per cent optical purity which is defined as the specific rotation of the enantiomeric mixture divided by specific rotation of one pure enantiomer times 100.

* The optical purity of the dihydrodiols formed from benzo[a]pyrene by rat liver microsomes from 3-methylcholanthrene-treated rats is taken from the data of Thakker et al. (14).
FIG. 2. HPLC separation of diastereomeric bis MTPA diesters of \([^3H]BP 4,5\)-dihydrodiol formed during the incubation of epoxide hydrase with \([^3H] (\pm )-BP 4,5\)-oxide (——). Similar separation for bis MTPA diesters of synthetic \((\pm )-BP 4,5\)-dihydrodiol is also shown (——). (See "Materials and Methods" for chromatographic conditions.) Peak a, \((\pm )-BP 4,5\)-dihydrodiol; Peak b, \((\pm )-BP 4,5\)-dihydrodiol.

FIG. 3. Proposed mechanism to explain the optical purities of BP 4,5- and 7,8-dihydrodiols formed by epoxide hydrase from the racemic arene oxides. The solid arrow (–→) designates front attack and the broken arrow (——) designates back attack by H₂O. Absolute stereochemistry of BP 4,5-dihydrodiol is not implied.
tained 7% 18O, indicating that 18O-enriched water in the incubation medium had attacked positions 4 and 5 of BP 4,5-oxide at relative rates of 3:2. The fact that the total 18O in the two phenol acetates (17%) does not equal that in the starting dihydrodiol (33%) suggests that some exchange of the dihydrodiol with solvent water had occurred during the course of the dehydration. However, comparable incorporation of 18O in positions 4 and 5 of racemic BP 4,5-oxide suggests that attack of water occurs at both positions to about the same extent. Moreover, it was also observed that when 30% or 75% of the racemic BP 4,5-oxide was metabolized to the corresponding dihydrodiol, similar optical purity (84% and 80%, respectively) was obtained. These results indicate that epoxide hydrase can utilize both enantiomers of BP 4,5-oxide as substrates and that it directs the attack of water at position 4 of one enantiomer and position 5 of the other enantiomer, predominantly only one of the chiral centers, giving rise to BP 4,5-dihydrodiol of high optical purity (Fig. 3).

The optical purity of BP 4,5-, 7,8-, and 9,10-dihydrodiol formed from the metabolism of benzo[a]pyrene by liver microsomes from rats treated with 3-methylcholanthrene were described earlier (14) and are listed in Table II. All three dihydrodiols formed by this route have very high optical purity (~92%, cf. Ref. 14). This result, along with the fact that epoxide hydrase forms dihydrodiols of low optical purity from racemic BP 7,8-oxide and racemic BP 9,10-oxide, suggests that these two oxides must be formed with high optical purity from RP by the monooxygenase system. Since the high optical purity of BP 4,5-dihydrodiol, formed by the microsomal metabolism of BP, could have resulted from the high stereospecificity of epoxide hydrase toward the racemic BP 4,5-oxide, it is not known whether or not the monooxygenase system metabolizes BP to the 4,5-oxide with high stereoselectivity.

Previous studies have indicated that the properties of rat liver epoxide hydrase are unaltered after pretreatment of the animals with either phenobarbital or 3-methylcholanthrene (19). However, the stereospecificity of the enzyme in microsomes obtained from induced or control animals has not been examined. Hence, the enantiomeric purities of BP 4,5- and 7,8-dihydrodiol obtained from the hydration of the corresponding arene oxide by microsomes from control, phenobarbital-, and 3-methylcholanthrene-pretreated rats as well as with purified epoxide hydrase from rats treated with phenobarbital were determined. The optical purity of the dihydrodiols obtained is compared in Table III. The enantiomeric purity of BP 4,5-dihydrodiol formed from racemic BP 4,5-oxide by epoxide hydrase from different microsomal sources and the homogeneous enzyme is identical, indicating that the high stereospecificity of the enzyme towards racemic BP 4,5-oxide is unaltered after pretreatment of rats with either phenobarbital or 3-methylcholanthrene. The hydration of racemic BP 7,8-oxide to BP 7,8-dihydrodiol occurred with low stereospecificity when epoxide hydrase from several sources was used (Table III).

BP 4,5-dihydrodiol, formed from the corresponding racemate, has much higher optical purity than the other two K region dihydrodiols (Tables II and III). It was of interest to determine if the high stereospecificity of epoxide hydrase was associated with the hydration of all K region arene oxides. The results in Table IV compare the enantiomeric purity of the K region dihydrodiols of benzo[a]pyrene, benz[a]anthracene and phenanthrene obtained from the corresponding racemic arene oxides. A steady decrease in the enantiomeric purity of the product was observed as the size of the substrate decreased. The enantiomeric purities of the metabolically formed K region dihydrodiols of benzo[a]pyrene, benz[a]anthracene, and phenanthrene were 78%, 42%, and 26%, respectively (Table IV).

**TABLE III**

| Stereoispecificity of purified epoxide hydrase and the membrane-bound enzyme obtained from control, phenobarbital-treated, and 3-methylcholanthrene-treated rats with benzo[a]pyrene 4,5- and 7,8-oxide as substrates |
|---------------------------------------------------------------|
| **Source of epoxide hydrase** | **Formation of (+)- and (-)-BP 4,5-dihydrodiol from racemic BP 4,5-oxide** | **Formation of (+)- and (-)-BP 7,8-dihydrodiol from racemic BP 7,8-oxide** |
| | % (+)-enantiomer | % (+)-enantiomer | % enantiomeric purity | % (+)-enantiomer | % (+)-enantiomer | % enantiomeric purity |
| Control | 90 | 10 | 80 | 54 | 46 | 8 |
| Phenobarbital microsomes | 89 | 11 | 78 | 54 | 46 | 8 |
| 3-Methylcholanthrene microsomes | 89 | 11 | 78 | 54 | 47 | 7 |
| Purified epoxide hydrase | 92 | 8 | 84 | 54 | 45 | 10 |

Values were calculated as described in Table II.

**DISCUSSION**

The stereospecific metabolism of polycyclic hydrocarbon arene oxides by rat liver microsomes or by highly purified epoxide hydrase has been examined. Several racemic K region and...
non-K region arene oxides were used as substrates for the enzyme, and the resulting dihydrodiols were analyzed for their enantiomeric purity by HPLC. The method of analysis consisted of esterification of the 14C-labeled dihydrodiols with optically pure MTPA to form diastereomers which were separated by HPLC. Radioactivity associated with each diastereomeric peak allows computation of the relative amount of each enantiomer present in the product. The accuracy of this radiochemical method of analysis compared to optical methods makes it ideally suited to stereochemical studies of this type where the amount of product is extremely limited.

Epoxide hydrase present in the microsomes from control, phenobarbital-treated, or 3-methylcholanthrene-treated rats as well as the enzyme purified to apparent homogeneity were found to be identical with regard to their stereospecificity, and these data suggest an identical mechanism of action of the enzyme from all four sources.

Epoxide hydrase appears to show higher stereospecificity toward the K region versus the non-K region arene oxides. Among the metabolically formed benzo[a]pyrene dihydrodiols, the 4,5-dihydrodiol has much higher enantiomeric purity (80%, Table II) than BP 7,8- and 9,10-dihydrodiol (8% and 22%, respectively, Table II). The low optical purity of BP 7,8-dihydrodiol appears to be due to the ability of epoxide hydrase to accept both enantiomers of the arene oxide as a substrate with almost equal ease. Since attack of H2O is directed almost exclusively at position 8, the R center of one enantiomer and the S center of the other is the point of attack with inversion. This leads to the formation of product with low optical purity. The preference for attack at C-8 on BP 7,8-oxide may be for both steric and electronic reasons. The lack of high specificity for either the S or R center at C-8 is unclear but parallels our previous results with naphthalene oxide (25). With BP 4,5-oxide, no positional specificity was demonstrated for the enzyme as judged by incorporation of 14C at positions 4 and 5, and the enzyme selects for only one of the chiral centers, presumably the S center (cf. Fig. 3) (18). As a result, both enantiomers of BP 4,5-oxide are hydrated to the same enantiomer of the dihydrodiol. The fact that from 30 to 75% of the substrate could be hydrated by epoxide hydrase without significant change in the optical purity of the product established that both enantiomers of BP 4,5-oxide are substrates for the enzyme. Other K region dihydrodiols, such as phenanthrene 9,10- and benz[a]anthracene 5,6-dihydrodiol are formed with less stereospecificity (36% and 49% optical purity, respectively) than is BP 4,5-dihydrodiol. Interestingly, the optical purity of the metabolically formed K region dihydrodiols increases with increasing size of the arene oxide substrate.

In conclusion, epoxide hydrase can have both very high positional specificity and stereospecificity toward arene oxides of different polycyclic aromatic hydrocarbons. The type and extent of specificity, however, appears to be governed by both steric and electronic factors associated with the substrate itself. The stereospecificity of cytochrome P-450-dependent monooxygenases and epoxide hydrase can play a major role in the metabolic activation of polycyclic aromatic hydrocarbons to mutagenic and carcinogenic metabolites. For example, the (−)- and (+)-enantiomers of BP 7,8-dihydrodiol undergo highly selective metabolism by the cytochrome P-450-dependent monooxygenase system to the diastereomeric BP 7,8-diol-9,10-epoxides. The (−)-enantiomer of BP 7,8-dihydrodiol is metabolized primarily to (+)-BP 7β,8α-diol-9α,10β-epoxide (4, 15) whereas the (+)-enantiomer is metabolized almost exclusively to (+)-BP 7α,8β-diol-9α,10α-epoxide (15). Both of the diastereomeric (+)-BP 7,8-diol-9,10-epoxides are highly mutagenic to bacterial and mammalian cells. (+)-BP 7β,8α-diol-9β,10β-epoxide is a more potent mutagen than (+)-BP 7β,8α-diol-9α,10α-epoxide in several strains of Salmonella typhimurium (11) while the reverse is true for the mutagenic activity of the diastereomers in Chinese hamster V-79 cells (10, 11, 26). The (−)-enantiomer of BP 7,8-dihydrodiol is 5 to 10-fold more tumorigenic to mouse skin than is the (+)-enantiomer suggesting that the BP 7β,8α-diol-9α,10α-epoxide is a potent carcinogen derived from BP (27). In light of the difference in chemical (11, 28) and biological activities of these diastereomeric diol epoxides (10, 11, 26, 29), the stereospecificity of the monooxygenase system and epoxide hydrase plays a pivotal role in determining the susceptibility of a particular tissue, organ, or species towards the carcinogenicity and toxicity of polycyclic aromatic hydrocarbons.

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