Characterization of Rat Lung Epoxide (Styrene Oxide) Hydrase with a Modified Radioactive Assay of Improved Sensitivity

Janeric Seidegård, Joseph W. DePierre, Maria S. Moron, Karin A. M. Johannesen, and Lars Ernster

INTRODUCTION

Polycyclic hydrocarbons may be a major cause of cancer in humans. A number of studies have demonstrated the carcinogenic properties of these compounds in different species, including man, and in different tissues, including the lung (2, 6, 12, 25, 27, 28, 32, 43, 52, 54, 57). In addition, polycyclic hydrocarbons have been observed to cause malignant transformations (5, 10) and mutagenesis (11) when applied to cells in culture.

The 1st step in the metabolism of polycyclic hydrocarbons by mammalian tissues is formation of arene oxides by the aryl hydrocarbon monooxygenase system, 1 component of which is cytochrome P-450 (44). There are a number of indications that these epoxide metabolites are considerably more mutagenic than the parent hydrocarbons themselves (1, 13, 24, 30, 31, 40). Such findings suggest that epoxide metabolites may be the immediate agents responsible for causing lung and skin cancer.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing approximately 180 to 200 g were used routinely. In several experiments the animals were given i.p. injections of methylchol-

SUMMARY

The epoxide hydrase assay developed by Oesch et al. (Biochim. Biophys. Acta, 227: 685-691, 1971) using [3H]styrene oxide as substrate was modified in three ways for use with rat lung microsomes: the substrate was purified before use, the volume of the incubation mixture was scaled down 4-fold, and the incubation time was extended to 45 min (activity was found to be linear for at least 60 min). These modifications increased the sensitivity of the assay procedure 75- to 150-fold. The procedure was found to be linear with lung microsomal protein up to at least 1.8 mg protein per incubation mixture.

This modified assay for epoxide hydrase was used to characterize the enzyme in rat lung. Its apparent Vₘₐₓ is 0.5 nmole of styrene glycol formed per min per mg microsomal protein, and its apparent Kₘ is 0.11 to 0.25 mM. The pH optimum is around 9.7. Upon subcellular fractionation of lung tissue, epoxide hydrase distributes in the same manner as a marker for the endoplasmic reticulum (reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase) and in a different way from markers for the nuclei, mitochondria, concentric lamellar organelles, lysosomes, Golgi membranes, plasma membrane, and soluble cytoplasm. The specific activity of epoxide hydrase in rough and smooth lung microsomes is about the same. Treatment i.p. of rats with methylcholanthrene (3 injections of 20 mg/kg), phenobarbital (5 daily injections of 80 mg/kg), or styrene oxide (5 daily injections of 40 mg/kg) did not induce lung microsomal epoxide hydrase activity. 1,1,1-Trichloro-2-propene 2,3-oxide was shown to be an uncompetitive inhibitor, and cyclohexene oxide was a noncompetitive inhibitor of this enzyme. Ethanol and butanol activate the epoxide hydrase of lung microsomes at low concentrations and inhibit it at higher concentrations.

Oesch and Bentley have concluded that benzopyrene oxide hydrase and styrene oxide hydrase activities are catalyzed by the same protein (47). This conclusion is based on the findings that the relative ratio of these activities in the preparation remains essentially unchanged during purification and that an antibody to the purified enzyme inhibits both benzopyrene oxide hydrase and styrene oxide hydrase in solubilized microsomes. As a result of this study, investigators who wish to examine arene oxide hydrase can do so using styrene oxide (which is relatively inexpensive and easily handled) as the substrate.

We have modified a published assay procedure for styrene oxide hydrase (49) in order to increase its sensitivity. This increased sensitivity has allowed us to characterize the epoxide hydrase of rat lung in various ways.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing approximately 180 to 200 g were used routinely. In several experiments the animals were given i.p. injections of methylchol-
anthanare (20 mg/kg body weight in corn oil, 5, 3, and 1 day before sacrifice), phenobarbital (80 mg/kg body weight in isotonic NaCl solution, once daily for 5 days preceding sacrifice), or styrene oxide (10, 20, or 40 mg/kg body weight in corn oil, once daily for 5 days preceding sacrifice). Control animals were inoculated with equal volumes of corn oil or isotonic NaCl solution. In most cases the rats were not starved before being killed by decapitation. However, in instances in which liver microsomes were prepared from the same animal whose lung was used, the rats were starved overnight (16 hr) before sacrifice. This starvation did not affect the recovery of lung microsomes or the level of epoxide hydrase found in lung microsomes.

**Chemicals.** 7-[3H]Styrene oxide (10 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, England. Cold styrene oxide was obtained from Schuchardt, Munich, Germany; cyclohexene oxide was obtained from Fluka AG Chemische Fabrik, Buchs, Switzerland; and 1,1,1,-trichloropropene 2,3-oxide was obtained from Aldrich-Europe, Beerse, Belgium. All other chemicals were obtained from commercial sources and were of reagent grade.

**Preparation of Microsomes.** Total, rough, and smooth lung microsomes were prepared by the method of Johanneisen et al. (35). The microsomes were resuspended in 0.25 M sucrose to a final concentration equivalent to 5 g wet lung tissue per ml, which gave a protein concentration of about 10 mg/ml. Total liver microsomes were prepared according to the method of Ernsten et al. (21).

**Subcellular Fractionation of Lung Tissue.** The tissue was subfractionated by the same procedure as that used by Matsubara and Tochino (42) to subfractionate rabbit lung.

**Enzyme Assays.** The procedures used here for determining enzyme activities were originally developed for tissues other than the lung. In order to avoid unnecessary assumptions, the linearity of these assays with enzyme amount and with time using lung preparations was investigated in an earlier study (35) for all of the enzymes except lactate dehydrogenase and found to be satisfactory. A similar check with lactate dehydrogenase has been carried out in the present study.

When enzymes that were not assayed in the presence of detergents were determined in whole homogenates or nuclear fractions, these preparations were sonically disrupted first to assure accessibility of added substrate to the enzyme. Sonic disruption for 1 or 2 min gave maximal activation for all of these activities, and almost all of them decreased if sonic disruption was continued beyond the time required to achieve maximal activity. When enzymes for which assays involved measuring inorganic phosphate were determined in particulate subcellular fractions isolated after homogenization of lung tissue in phosphate buffer, these fractions were first washed once with and then resuspended in 0.15 M Tris-Cl, pH 7.5.

NADPH-cytochrome c reductase was measured at 30° by monitoring the reduction of cytochrome c spectrophotometrically at 550 nm as described by Dallner (14). For reasons discussed by Johanneisen et al. (35), this assay should be started by the addition of NADPH rather than by the addition of cytochrome c or lung preparations and corrected for the rate of cytochrome c reduction occurring before NADPH addition. With isolated, washed microsomal fractions this correction is negligible.

Succinate-cytochrome c reductase (56), acid p-nitrophenyl phosphatase (35), acid β-glycerophosphatase (35), p-nitrophenyl mannosidase (20), AMPase (35), and lactate dehydrogenase (53) were assayed using published procedures.

**Chemical Measurements.** Protein was determined using the method of Lowry et al. (38) with bovine serum albumin as standard. Phospholipid (15), DNA (8), and RNA (9) were determined using published procedures.

**Miscellaneous Procedures.** Scintillation counting was carried out on a Packard Tri-Carb Model 2420 liquid scintillation spectrophotometer using Tritosol scintillation fluid (22). Sonic disruption was carried out on a Branson sonicator at a setting of 1.5 amp. The sample holders were immersed in ice water to prevent excessive heating.

**RESULTS**

**Assay of Epoxide Hydrase.** The procedure used for measuring epoxide hydrase activity was essentially that developed by Oesch et al. (49) for liver microsomes. However, this procedure had to be modified in 3 ways in order to make it sensitive enough for routine use with rat lung preparations.

First, the substrate was purified before use. One μl of the undiluted [3H]styrene oxide was dissolved in 1.5 ml hexane, and this solution was extracted 3 times with 100 μl 0.5 M Tris-Cl, pH 7.5. The [3H]styrene oxide was then extracted from the hexane into 1.5 ml acetonitrile, and this procedure was repeated twice. Of the original radioactivity, 65 to 80% was recovered in the combined acetonitrile aliquots, and unlabeled styrene oxide was added to give an 80 mM substrate solution with a specific radioactivity of 1.3 mM/μmol. As can be seen from Chart 1, the background after 45 min of incubation (the standard incubation time; see below) is decreased about 2.5-fold by purification of the [3H]styrene oxide. The substrate solution was stored at −18°, and the background was found to increase upon prolonged storage. Repurification was required after several months in the freezer.

It is also apparent from Chart 1 that the background with purified substrate is linear with time for at least 1 hr and is independent of the presence or absence of boiled micro-
somal protein. We suspected that this remaining background is due to the nonenzymatic hydrolysis of styrene oxide. Indeed, when the background radioactivity was extracted into ethyl acetate and subjected to thin-layer chromatography on silica gel using chloroform:ethyl acetate, 1:1, as the developing solution, a single spot with an Rf the same as that of styrene glycol was found. If the remaining background is indeed styrene glycol, it would seem to be virtually impossible to lower this background further.

The 2nd modification was to scale down the 0.4-ml incubation volume used with liver microsomes to 0.1 ml. Thus, the details of the assay were: 25 μl 0.5 M Tris, pH 7.5, and 75 μl microsomes (usually containing about 0.75 mg protein) or other fractions were mixed in a glass-stoppered tube and preincubated at 37° for 1 or 2 min. The reaction was started by addition of 2 μl of the purified substrate solution (containing 160 nmoles of [3H]styrene oxide). After incubation (usually for 45 min) at 37°, the reaction was terminated by adding ice-cold petroleum ether (b.p. 40-60°) and shaking vigorously on a Vortex mixer for 5 min. After separation of the 2 phases by spinning in a desk centrifuge for 5 min, the tubes were placed in a freezer to allow the lower water phase to freeze, and the upper petroleum ether phase was subsequently decanted. This extraction with petroleum ether was repeated once, and then the product styrene glycol was extracted using 1 ml ethyl acetate in an essentially identical manner (without the freezing step). A 100-μl aliquot of the combined petroleum ether extracts and a 400-μl aliquot of the ethyl acetate phase were counted and corrected for quenching. Measurements were routinely carried out in duplicate, and the duplicates normally agreed within 10% or less.

A few comments on this procedure are necessary. In the first place the assay is performed at pH 7.5, even though the pH optimum of the enzyme is considerably higher (see below). The lower pH, which gives an activity approximately 70% of that obtained at the pH optimum, was chosen in order to simulate in vivo conditions. Second, Oesch et al. (49) routinely assay epoxide hydrase in the presence of 1% Tween 80 in order to obtain a higher activity. However, the use of Tween 80 in Tris buffer either at pH 7.5 or 9.0 has no effect on rat lung epoxide hydrase. Finally, it was found that the presence of up to 15% acetonitrile during the reaction does not affect the activity of lung epoxide hydrase. Addition of 2 μl of substrate solution results in a 2% solution of acetonitrile in the incubation mixture.

Epoxide hydrase activity measured using a 0.1-ml incubation volume is identical to that using a 0.4-ml volume. This assay of epoxide hydrase with lung microsomes is linear for at least 60 min. Thus, the 3rd modification made in the assay procedure was to incubate for 45 min instead of 5 min, the time period used with liver microsomes. However, when such long incubation times were used, a net loss of radioactivity was noticed. This loss, which is presumably due to evaporation of styrene oxide, could be prevented by carrying out the assay in glass-stoppered tubes (Chart 2).

These 3 modifications increase the sensitivity of the assay for epoxide hydrase 75- to 150-fold over the procedure developed with liver microsomes. As a result, using rat lung microsomes, a 15- to 20% conversion of substrate into product over a 2 to 3% background is normally obtained.

The present assay procedure for epoxide hydrase is linear up to at least 1.8 mg lung microsomal protein per incubation mixture. In addition, the activity seems to be reasonably stable in the freezer. No decrease in activity is seen during the 1st 2 days at −18°, and a week after freezing the activity is still 85% of the original activity. All of our experiments were carried out with fresh microsomes or microsomes that had been frozen for only a few days.

**Apparent Km and Vmax of Lung Epoxide Hydrase.** The dependence of lung microsomal epoxide hydrase activity on substrate concentration is shown in Chart 3. A smooth line has been drawn, but there are indications of a preliminary plateau between 0.5 and 2.0 mM styrene oxide before the activity rises to its final apparent Vmax of about 0.5 n mole styrene glycol formed per min per mg lung microsomal protein. Many factors might be involved in the appearance of such a plateau in this complicated system containing at least 2 phases (the hydrophobic phase of the microsomal membrane and the aqueous phase) and a substrate that is only slightly soluble in water. With these reservations, if the data in Chart 3 are arranged in a Lineweaver-Burk plot (37), an apparent Km for styrene oxide of 0.11 to 0.25 mM is obtained. The 1.6 mM concentration of styrene oxide used in the present assay procedure thus seems to be appropriate.

**pH Dependence.** Lung epoxide hydrase activity was investigated over a wide range of pH (Chart 4). Three different buffers, phosphate, Tris, and glycine, were used, and no individual buffer effects were observed.

**Subcellular Localization.** In order to investigate the subcellular localization of epoxide hydrase in rat lung, the tissue was subfractionated as described under "Materials and Methods." The cellular localization of epoxide hydrase in rat lung, the tissue was subfractionated as described under "Materials and Methods."
and Methods," and the distribution of epoxide hydrase was compared to the distribution of various marker enzymes. The markers used were: DNA for the nuclei, succinate-cytochrome c reductase for mitochondria, acid p-nitrophenyl phosphatase for concentric lamellar organelles, acid β-glycerophosphatase for lysosomes, NADPH-cytochrome c reductase for the endoplasmic reticulum, p-nitrophenylmannosidase for the Golgi apparatus, AMPase for the plasma membrane, and lactate dehydrogenase for the soluble cytoplasm. [A detailed discussion of this choice of markers is given by Johannesen et al. (35).]

It can be seen from the de Duve plots shown in Chart 5 that the distribution of epoxide hydrase can be clearly distinguished from that of all other markers except for NADPH-cytochrome c reductase. The difference between the distributions of epoxide hydrase and AMPase is not fully so obvious as the other differences. However, it can be calculated from Chart 5 that the ratio of the relative specific activity in the microsomal fraction to the relative specific activity in the mitochondrial fraction is 1.6 for epoxide hydrase and 2.5 for AMPase; the corresponding value for NADH-cytochrome c reductase is 1.7. It can also be seen that RNA distributes in a manner similar to that in which epoxide hydrase and NADPH-cytochrome c reductase distribute; this is presumably due to the ribosomes bound to the endoplasmic reticulum. Finally, it is interesting to note that the subcellular distribution of all of these enzymes in lung tissue is consistent with their being localized on the organelles for which they have been respectively used as markers.

As additional evidence that epoxide hydrase and NADPH-cytochrome c reductase are localized on the same organelle in rat lung, the mitochondrial plus microsomal fractions were divided into 5 fractions by differential centrifugation. Chart 6A illustrates the close correspondence between the distributions of epoxide hydrase and NADPH-cytochrome c reductase in these subfractions, whereas the distributions of succinate-cytochrome c reductase and p-nitrophenylmannosidase are quite different. Chart 6B confirms that the ratio of the specific activity of epoxide hydrase to the specific activity of NADPH-cytochrome c reductase is almost the same in all 5 of these subfractions. It can be concluded that epoxide hydrase is localized on the endoplasmic reticulum in rat lung.

Table 1 shows that epoxide hydrase is distributed between rough and smooth microsomes from rat lung in approximately the same manner as NADPH-cytochrome c reductase.

Attempts to Induce Epoxide Hydrase Activity in the
Lung. The synthesis of arene oxides by the cytochrome P-450 system can be induced 10- to 20-fold in liver and lung by i.p. administration of methylcholanthrene to rats (19, 26, 29, 35, 36, 59). This activity is also inducible in liver, although to a much lesser extent, by phenobarbital (23). Table 2 demonstrates that treatment of rats either with methylcholanthrene or with phenobarbital at the doses stated had no effect on lung epoxide hydrase activity, although in these same rats methylcholanthrene induced liver benzpyrene monooxygenase activity 16-fold and phenobarbital induced liver NADPH-cytochrome c reductase activity 4- to 5-fold.

Table 2 also documents an attempt to bring about "substrate induction" of epoxide hydrase by i.p. administration of styrene oxide in corn oil to rats. This treatment had no significant effect on epoxide hydrase, NADPH-cytochrome c reductase, or DT-diaphorase activities in the lung and liver and no effect on cytochromes P-450 and b5 in liver microsomes. Even the highest dose of styrene oxide did not have any apparent toxic effects on the animals.

Inhibitors. Cyclohexene oxide and 1,1,1-trichloropropene 2,3-oxide are known to be noncompetitive and uncompetitive inhibitors, respectively, of hepatic epoxide hydrase with respect to the substrate styrene oxide (50). Chart 7 illustrates that lung microsomal epoxide hydrase is affected by these 2 inhibitors in the same manner as the liver enzyme. An apparent $K_i$ for cyclohexene oxide of 80 $\mu$M could be calculated for the lung enzyme. Other experiments showed that 40 to 50 $\mu$M 1,1,1-trichloropropene 2,3-oxide inhibits the saturated lung epoxide hydrase approximately 50%.

Effect of Alcohols. Oesch et al. (50) have demonstrated that liver microsomal epoxide hydrase is activated by low concentrations of 1-alkanols and inhibited by higher concentrations. Chart 8 shows that this is also the case for lung microsomal epoxide hydrase. Ten % ethanol (v/v) (1.7 M) is seen to activate the lung enzyme approximately 100%, while inhibition is seen above 18% ethanol (v/v). Butyl alcohol has only a small activating effect at 5% (v/v) (0.55 M) and inhibits potently at higher concentrations.

**DISCUSSION**

Three modifications have been made in the standard radioactive assay procedure for epoxide (styrene oxide) hydrase: the substrate is purified before use, the volume of the incubation mixture is scaled down 4-fold, and the incubation time is lengthened 9- to 12-fold (to 45 to 60 min). These changes increase the sensitivity of the assay procedure 75- to 150-fold, thus providing a reliable and convenient method for characterizing epoxide hydrase in tissues where its activity is quite low, such as rat lung.

Since polycyclic hydrocarbons appear to cause cancer much more easily in lung than in liver, and since epoxide hydrase may play an important role in the etiology of such cancer, it is of interest to compare the properties of epoxide hydrase in the two tissues.

### Table 1

<table>
<thead>
<tr>
<th>Microsomes</th>
<th>NADPH-cytochrome c reductase</th>
<th>Epoxide hydrase</th>
<th>Hydrase: reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>56.6</td>
<td>422</td>
<td>7.46</td>
</tr>
<tr>
<td>Rough</td>
<td>85.4</td>
<td>516</td>
<td>6.04</td>
</tr>
<tr>
<td>Smooth</td>
<td>46.7</td>
<td>371</td>
<td>7.94</td>
</tr>
</tbody>
</table>

* The preparation of rough and smooth lung microsomes was as described under "Materials and Methods."
* $\mu$moles cytochrome c reduced per min per mg microsomal protein.
* pmoles styrene glycol formed per min per mg microsomal protein.

### Table 2

<table>
<thead>
<tr>
<th>Treatment of animals</th>
<th>N</th>
<th>pmoles styrene glycol formed/min/mg protein</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl solution</td>
<td>20c</td>
<td>0.457 $\pm$ 0.040</td>
<td>100</td>
</tr>
<tr>
<td>Phenobarbital d</td>
<td>30c</td>
<td>0.425 $\pm$ 0.032</td>
<td>93</td>
</tr>
<tr>
<td>Corn oil</td>
<td>15c</td>
<td>0.510 $\pm$ 0.017</td>
<td>112</td>
</tr>
<tr>
<td>Methylcholanthrene</td>
<td>15c</td>
<td>0.445 $\pm$ 0.034</td>
<td>97.4</td>
</tr>
<tr>
<td>Styrene oxide f</td>
<td>2</td>
<td>0.566</td>
<td>124</td>
</tr>
</tbody>
</table>

* By i.p. injection.
* The figures given represent means $\pm$ the average deviation.
* The animals were pooled into groups of 5 for the preparation of lung microsomes and the measurement of epoxide hydrase activity.
* Phenobarbital, 80 mg/kg, administered in NaCl solution once daily for 5 days before sacrifice.
* Methylcholanthrene, 20 mg/kg, administered in corn oil, 5, 3, and 1 day before sacrifice.
* Styrene oxide, 40 mg/kg, administered in corn oil once daily for 5 days before sacrifice.
J. Seidegård et al.

hydrase in these 2 organs. Table 3 shows that these properties are quite similar. In both liver and lung, the apparent $K_m$ of the enzyme for styrene oxide is about 0.2 to 0.3 mM. The corresponding value for purified human liver epoxide hydrase is 0.38 mM (51). The pH optimum of epoxide hyderase in both organs is quite alkaline, as it also is for purified human liver epoxide hydrase (pH optimum = 9; Ref. 51).

On the basis of a preparative study, Oesch et al. (49) suggested that epoxide hydrase is localized on the endoplasmic reticulum in the livers of guinea pigs, rats, and rabbits. Oesch (44) has even proposed that this enzyme could be used as a marker for the endoplasmic reticulum. The analytical subcellular fractionation of rat lung carried out in the present study is strong evidence that epoxide hydrase is localized on the endoplasmic reticulum in this tissue as well.

1,1,1-Trichloropropene, 2,3-oxide, and cyclohexene oxide inhibit lung epoxide hydrase in the same way they inhibit the liver enzyme, and ethanol and butyl alcohol have the same effect on these 2 enzymes. The only differences, other than in specific activity, are that Tween 80 stimulates the liver but not the lung enzyme and that phenobarbital induces epoxide hydrase activity in the liver but not in the lung. This latter finding is consistent with previous reports that i.p. injection of phenobarbital greatly increases the levels of NADPH-cytochrome c reductase, cytochrome P-450, and drug-metabolizing activity in rat liver but affects none of these components in rat lung (41).

Of particular interest in terms of cancer caused by polycyclic hydrocarbons are the relative activities of epoxide hydrase in liver and lung. Table 4 gives these values on the basis of a number of different parameters, as well as presenting values for benzopyrene monooxygenase and for the ratios of benzopyrene monooxygenase to epoxides hydrase activities in rat liver and lung. Although many of the values in this table are only approximate, and although activity of epoxide hydrase with arene oxides as substrate is substantially lower than styrene oxide hydrase activity (48, 50), some interesting conclusions can be drawn from this table.

First, both epoxide hydrase and benzopyrene monooxygenase activities are substantially lower in rat lung tissue than in rat liver. Thus, the lung might be expected to play a major quantitative role in the metabolism of polycyclic hydrocarbons only when lung tissue is exposed to these compounds before the liver, e.g., when polycyclic hydrocarbons are inhaled.

Second, the ratio of epoxide hydrase to benzopyrene monooxygenase activity in rat liver is about 3 times higher than in rat lung. Finally, the ratio of epoxide hydrase to benzopyrene monooxygenase activity is much lower in both liver and lung after treatment of the animals with methylcholanthrene. It is difficult to relate these findings directly to the fact that the lung is more susceptible to the carcinogenic effects of polycyclic hydrocarbons than is the liver. Benzopyrene monooxygenase catalyzes the formation of the apparently dangerous arene oxides, while epoxide hydrase participates in the further metabolism of these oxides to less dangerous products. However, as discussed under "Introduction," the diols produced by epoxide hydrase may be further metabolized in the cytochrome P-450 system to diol epoxides, which may be even more dangerous than the original arene oxide because they are more stable, are less readily hydrated by epoxide hydrase or conjugated with glutathione, and/or for other reasons.

<table>
<thead>
<tr>
<th>Property</th>
<th>Liver</th>
<th>Rat lung*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity in microsomes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5</td>
</tr>
<tr>
<td>Apparent $K_m$ (mM)</td>
<td>0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.11–0.25</td>
</tr>
<tr>
<td>pH optimum</td>
<td>8.7–9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.7</td>
</tr>
<tr>
<td>Subcellular localization</td>
<td>Endoplasmic reticulum</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>Inducibility with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylencholanthrene</td>
<td>None&lt;sup&gt;p&lt;/sup&gt;</td>
<td>None</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>300% of Control&lt;sup&gt;f&lt;/sup&gt;</td>
<td>None</td>
</tr>
<tr>
<td>Styrene oxide</td>
<td>None&lt;sup&gt;p&lt;/sup&gt;</td>
<td>None</td>
</tr>
<tr>
<td>Inhibition with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,1,1-Trichloropropene, 2,3-oxide</td>
<td>Uncompetitive&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Uncompetitive</td>
</tr>
<tr>
<td>Cyclohexene oxide</td>
<td>Noncompetitive&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Noncompetitive</td>
</tr>
<tr>
<td>Effect of Ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activates at low concentration, inhibits at high concentration&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Activates at low concentration, inhibits at high concentration&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Butanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>Activates&lt;sup&gt;f&lt;/sup&gt;</td>
<td>None</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values from present study.

<sup>b</sup> nmoles styrene glycol formed per min per mg protein.

<sup>c</sup> An approximate value for rat liver microsomes representing a rough average of the values from Refs. 3, 4, 16, 33, 39, 46, and 47.

<sup>d</sup> Rat liver microsomes. See Ref. 3.

<sup>e</sup> Epoxide hydrase purified from rat liver. See Refs. 4 and 39.

<sup>f</sup> Guinea pig liver. See Refs. 44 and 45.

<sup>p</sup> Our own results (not presented).

<sup>h</sup> Epoxide hydrase purified from guinea pig liver. See Ref. 44.
Comparison of the levels of epoxide hydrase and benzpyrene monooxygenase activities in rat liver and lung

<table>
<thead>
<tr>
<th>Property</th>
<th>Rat liver</th>
<th>Rat lung</th>
<th>Liver/lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet wt (g)</td>
<td>6.9*</td>
<td>1.37*</td>
<td></td>
</tr>
<tr>
<td>Endoplasmic reticulum mg protein/g wet wt in isolated microsomes</td>
<td>23*</td>
<td>1.62*</td>
<td></td>
</tr>
<tr>
<td>Recovery of NADPH-cytochrome c reductase in isolated microsomes</td>
<td>60%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Total mg protein/g wet wt*</td>
<td>38.3</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Epoxide hydrase Specific activity* in isolated microsomes</td>
<td>8'</td>
<td>0.5*</td>
<td>16</td>
</tr>
<tr>
<td>Total activity/g wet wt*</td>
<td>306</td>
<td>4.75</td>
<td>64</td>
</tr>
<tr>
<td>Total activity/organ/</td>
<td>2111</td>
<td>6.51</td>
<td>324</td>
</tr>
<tr>
<td>Benzpyrene monooxygenase Specific activity* in isolated microsomes</td>
<td>0.5</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.0*</td>
<td>0.6*</td>
<td>6.7</td>
</tr>
<tr>
<td>Total activity/g wet wt*</td>
<td>19.2</td>
<td>0.95</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>153</td>
<td>5.7</td>
<td>27</td>
</tr>
<tr>
<td>Total activity/organ/</td>
<td>132</td>
<td>1.30</td>
<td>102</td>
</tr>
<tr>
<td>Control</td>
<td>1056</td>
<td>7.81</td>
<td>135</td>
</tr>
<tr>
<td>Ratio of epoxide hydrase:benzpyrene monooxygenase activity*</td>
<td>16</td>
<td>5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* Mean of 10 determinations; our data (not published).
* See Ref. 35.
* See Ref. 18.
* mg protein in isolated microsomes × recovery.
* nmol styrene glycol formed per min per mg microsomal protein.
* See Table 3, Footnote c.
* From the present study.
* Specific activity × total mg microsomal protein per g wet weight.
* Total activity per g wet weight × g wet weight per organ.
* nmol benzpyrene metabolized per min per mg microsomal protein.
* A rough average of the values from Refs. 19, 26, 29, 36, and 59.
* A rough average of the values from Refs. 26, 35, and 59.
* With methylcholanthrene.
* See Ref. 19.
* See Ref. 59.
* This ratio is the same when calculated using specific activities, total activities per g wet weight, or total activities per organ.

REFERENCES

chemical Analysis of Membranes, pp. 73-131. London, England: Chap- 
mann and Hall, 1976.
Reliable, Sensitive, and Convenient Radioassay for Benzpyrene 
21. Ernst, L., Siekevitz, P., and Palade, G. E. Enzyme-Structure Relations-
ships in the Endoplasmic Reticulum of Rat Liver. J. Cell Biol., 15: 541- 
562, 1962.
22. Fricke, U. Tritosol: A New Scintillation Cocktail Based on Triton X-100. 
23. Gnossapellus, U., Thor, H., and Orrenius, S. A Comparative Study on the 
Effects of Phenobarbitalt and 3,4-Benzpyrene on the Hydroxylating En-
zyme System of Rat-Liver Microsomes. Chem.-Biol. Interactions, 1: 125- 
137, 1969/70.
24. Grover, P. L., Sims, P., Huberman, H., Kuroki, T., and 
Heidelberger, C. In Vitro Transformation of Rodent Cells by K-Region 
1098-1101, 1971.
25. Hanno, M. G., Nettlesheim, P., and Gilbert, J. R. (eds.), Inhalation Car-
ion, Division of Technical Information, 1970.
Hydroxylation to Phenol by Pulmonary and Hepatic Microsomes. Drug 
Tract Tumor in Hamsters Induced by Benzo(a)pyrene. Cancer Res., 33: 
Tract Tumor in Hamsters Induced by Benzo(a)pyrene. Cancer Res., 33: 
Tract Tumor in Hamsters Induced by Benzo(a)pyrene. Cancer Res., 33: 
Mutagenicity to Mammalian Cells of Epoxides and Other Derivatives of 
1971.
31. Huberman, E., Kuroki, T., Marquardt, M., Selkirk, J. K., Heidelberger, C., 
Grover, P. L., and Sims, P. Transformation of Hamster Embryo Cells by 
Epoxides and Other Derivatives of Polycyclic Hydrocarbons. Cancer 
32. Inui, N., and Takayama, S. Effect of Cigarette Tar Upon Tissue Culture 
33. James, M. G. O., Fouts, J. R., and Bend, J. R. Hepatic and Extrhepatic 
Metabolism, in Vitro, of an Epoxide (8-4-C-O-Styrene oxide) in the Rabbit. 
34. Jarina, D. M., and Daly, J. W. Arine OXides: A New Aspect of Drug 
35. Johannessen, K., DePierre, J. W., Bergstrand, A., Dallner, G., and 
Ernest, L. Preparation and Characterization of Total, Rough, and 
Smooth Microsomes from the Lung of Control and Methylcholanthrene 
36. Leutz, J. C., and Gelbion, H. V. Benzo(a)pyrene-4,5-Oxide Hydratase: 
Assay, Properties, and Induction. Biochem. Pharmacol., 23: 672- 
725, 1975.
37. Lineweaver, H., and Burd, D. The Determination of Enzyme Dissociation 
Measurement with the Folin Phenol Reagent. J. Biol. Chem., 193: 265- 
275, 1951.
40. Marquardt, M., Kuroki, T., Huberman, E., Selkirk, J. K., Heidelberger, C., 
Grover, P. L., and Sims, P. Malignant Transformation of Cells Derived 
from Mouse Prostate by Epoxides and Other Derivatives of Polycyclic 
41. Matsubara, T., Prough, R. A., Burke, M. D., and Estabrook, R. W. The 
Preparation of Microsomal Fractions of Rodent Respiratory Tract and 
42. Matsubara, T., and Tochin, Y. Electron Transport System of Lung 
Microsomes and Their Physiological Functions. J. Biochem., 70: 981- 
991, 1971.
Center.
44. Oesch, F. Mammalian Epoxide Hydrases: Inducible Enzymes Catalysing 
the Inactivation of Carcinogenic and Cytoxic Metabolites Derived from 
45. Oesch, F. Transpalacental Control of Epoxide Hydratase and Its Relation-
ship to the Control of Microsomal Monoxygenase. Federation Euro-
46. Oesch, F. Differential Control of Rat Microsomal "Aryl Hydrocarbon." 
Monoxygenase and Epoxide Hydratase. J. Biol. Chem., 251: 79-87, 
1976.
47. Oesch, F., and Bentley, P. Antibodies against Homogeneous Epoxide 
Hydratase Provide Evidence for a Single Enzyme Hydrating Styrene 
Epoxide Hydrase in Microsomes and in a Purified Preparation: Evidence 
49. Oesch, F., Jerina, D. M., and Daly, J. A Radiometric Assay for Hepatic 
Epoxide Hydratase Activity with 7-4-Styrene Oxide. Biochem. Biophys. 
50. Oesch, F., Kaubisch, N., Jerina, D. M., and Daly, J. W. Hepatic Epoxide 
Hydratase: Structure-Activity Relationships for Substrates and Inhibitors. 
51. Oesch, F., and Thoenen, H. Epoxide Hydrase in Human Liver Biopsy 
1974.
52. Oesch, F., and Thoenen, H. Epoxide Hydrase in Human Liver Biopsy 
1974.
53. Oesch, F., and Thoenen, H. Epoxide Hydrase in Human Liver Biopsy 
1974.
54. Oesch, F., and Thoenen, H. Epoxide Hydrase in Human Liver Biopsy 
1974.
55. Oesch, F., and Thoenen, H. Epoxide Hydrase in Human Liver Biopsy 
1974.
Characterization of Rat Lung Epoxide (Styrene Oxide) Hydrase with a Modified Radioactive Assay of Improved Sensitivity
