COMPREHENSIVE TOXICOLOGY

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Volume 3
Biotransformation

Volume Editor
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PERGAMON

3.18
Epoxide Hydrolases

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3.18.1 INTRODUCTION

Epoxide hydrolases (EHs) are enzymes that add water to three-membered cyclic ethers. Usually the products are 1,2 or vicinyl diols. In the case of epoxides on aromatic systems the products often are termed dihydrodiols to distinguish them from catechols. Several epoxide hydrolases are described in mammals including cholesterol epoxide hydrolase which hydrates the 5,6-oxide of cholesterol and other Δ^5-steroid epoxides,[1] leukotriene A_4 hydrolase which converts this 5,6-epoxide to the 5,12-diol, also known as leukotriene B_4 or leukocyte aggregating factor,[2] and hepoxilin hydrolase.[3] This chapter concentrates on the soluble and microsomal epoxide hydrolases (sEH and mEH) because both enzymes hydrate a wide range of substrates consistent with a role in xenobiotic metabolism. Previous names include epoxide hydrases and epoxide hydratases, but the current name of epoxide hydrolases (E.C. 3.3.2.3) is consistent both with their known mechanism of catalysis and their suspected evolution.

An epoxide is a three-membered cyclic ether with ethylene oxide the simplest member of the series. These are often described as oxides of the corresponding alkene, such as trichloropropene oxide. Many reactions of other ethers are common to epoxide-containing compounds, but they also have unique properties not shared by acyclic ethers or cyclic ethers on larger ring systems. The oxygen–carbon bonds are highly polarized. This polarization with the highly strained ring leads many epoxides to be exceptionally reactive. The electron density of the oxygen is surprisingly low, indicating that the unshared electron pairs are pulled back into a ring with an aromatic nature. Thus, like a cyclopropane ring, an epoxide ring can conjugate with other groups if their *p* orbitals can orient themselves in the same plane as the epoxide ring.[4] The high reactivity of the epoxide ring on some compounds leads to it being responsible for the electrophilic attacks of many of the most potent known mutagens, toxins, and carcinogens. Molecules that have an affinity for critical biological targets and contain a reactive epoxide can be very dangerous. However, some epoxides are so reactive that they are degraded rapidly and present no risk to biological systems. Other epoxides, such as heptachlor epoxide or tridiphane, can be extraordinarily stable in both chemical and biological systems due to steric hindrance and/or electronic properties.[5,6] Most epoxides are of intermediate reactivity. They are not so reactive as to pose acute risk to biological nucleophiles, but neither are they environmentally stable moieties. Epoxide hydrolases can be seen to complement glutathione transferases in the conversion of epoxides to more polar metabolites and thus their elimination.

Possible endogenous roles in chemical mediation for the EHs are discussed below, but these enzymes have an important role in toxicology. Not only are epoxide-containing compounds ubiquitous components of our diet from both natural and man-made sources, but a variety of aromatic and alkenic compounds are metabolized to epoxides endogenously. Sometimes these intermediate epoxides are highly toxic due to their reactivity. Even the diols can be toxic or metabolized to derivatives of high toxicity. However, the overall pathway from an alkenic or aromatic system to an epoxide and diol not only greatly increases the water solubility of the metabolite and dramatically increases its rate of clearance, but it provides groups that can be conjugated for still more rapid elimination. Thus, epoxide hydrolases are key enzymes in the lipophile to hydrophile conversion critical to survival of organisms. Numerous studies have shown that both the soluble and microsomal EHs are capable of reducing mutagenicity and cytotoxicity in a variety of systems.

EHs occur in numerous organisms. For instance, sEHs have been cloned and expressed from both the potato (*Solanum tuberosum*)[7] and the mouse eared cress (*Arabidpsis thaliana*).[8] Possible roles for the enzymes include fatty acid oxide metabolism, metabolism of phytoalexins, and metabolism to catins and suberins.[9] In insects, epoxide hydrolases are responsible for the catabolism of a key chemical mediator, juvenile hormone,[10] and they may provide defense against a plethora of plant defensive compounds.[11] However, this chapter will emphasize mammalian systems. There are numerous reviews in this area. Oesch[12] provided an early comprehensive review of the literature on mEH while Hammock *et al.*[13] covered sEH. Other reviews emphasizing the mEH include those by Brooks,[5] Guengerich,[14] and Lu and Miwa,[15] while Wixtrom and Hammock[16] provide a comprehensive comparison of the mEH and sEH and concentrate on analytical methods.

3.18.2 SUBSTRATE SELECTIVITY

3.18.2.1 Endogenous Substrates

It is not known if either the vertebrate mEH or the sEH have endogenous substrates or what these substrates are. The mEH will hydrate compounds, such as 16α,17α-epoxyandrostan-3-one, but a possible physiological role is still

question.[17,18] The soluble epoxide hydrolase will hydrate squalene oxide, squalene dioxide, lanosterol epoxide, and related compounds with a low K_m suggesting that they may be endogenous substrates.[16] These oxygenated steroids may be involved in regulation of cholesterol biosynthesis or in atherosclerosis. The enzyme hydrates *cis*- and *trans*-fatty acid epoxides with a high rate and a degree of regio- and stereospecificity, suggesting a possible endogenous role.[19–31] Adjacent diepoxides are converted to unique fatty acid tetrahydrofuran diols.[23,24] Degradation of fatty acid epoxides, which can form metabolically and are common in food, is important. If epoxides or diepoxides of fatty acids, such as those of arachidonic acid, are established as chemical mediators, the sEH could be involved in their degradation. Similarly, these epoxides could be intermediates on a pathway to biologically active diols as has been shown for the leukotoxins (epoxides of linoleic acid).[32,33]

3.18.2.2 Surrogate Substrates

If interested in the rate of hydration of a particular compound, it is best to use that compound as the substrate for monitoring the enzyme activity. However, when comparing enzyme activities, there are a variety of surrogate or diagnostic substrates in routine use that facilitate comparison with literature values. It must be cautioned that these substrates have been optimized, based upon data primarily from a single species and tissue. Thus, controls should be performed as they are applied to other situations. With both the mEH and sEH, it is becoming clear that even very minor variations in the primary structure of the enzyme can result in major changes in substrate selectivity. A useful pair of substrates that allow simple comparison with literature values include *cis*-stilbene oxide to monitor the mEH at basic pH and *trans*-stilbene oxide to monitor the sEH at neutral pH (Figure 1).

3.18.2.3 Structure–Activity Relationships

Since the mid-1980s, few extensive comparisons have been made for the substrate selectivity of the mEH and sEH. Thus, earlier reviews provide a good summary of the primary literature.[12–16] Unfortunately these earlier studies were usually done only with rodent

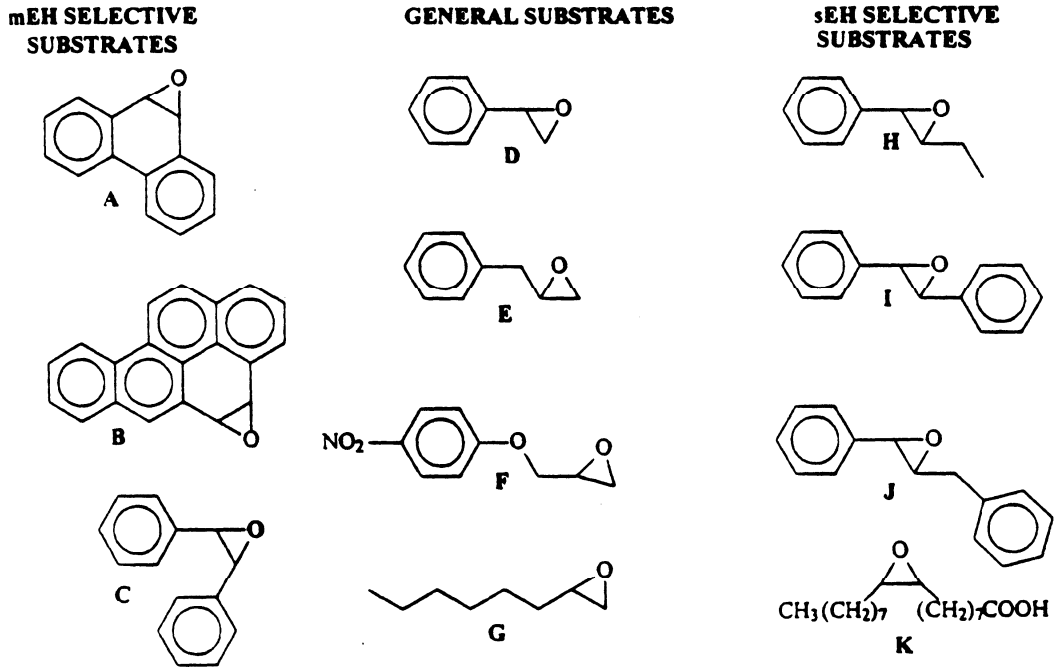


Figure 1 Common substrates for analysis of EHs. Selective substrates for mEH include (A) phenanthrene oxide, (B) benzo(*a*)pyrene 4,5-oxide, and (C) *cis*-stilbene oxide. The monosubstituted epoxides (D) styrene oxide, (E) allyl benzene oxide, (F) 4-nitrophenyl glycidyl ether, and (G) octene oxide are usually are substrates for both enzymes. *trans*-β-Substituted styrene oxides including (I) *trans*-stilbene oxide and (J) *trans*-β-benzyl styrene oxide are selective substrates for sEH. (K) The sEH also hydrates both *cis* and *trans* fatty acid epoxides.

enzyme, the enzymes were not of absolute purity, a very limited series of compounds were used and commonly true substrate specificity in terms of k_{cat}/K_m ratios were not determined. Hopefully these studies will be repeated with recombinant human enzymes in sufficient detail to map the catalytic site and allow prediction of substrate preferences.

Both the mEH and sEH seem to have one or more lipophilic binding sites. Arene oxides seem to be hydrated largely by the mEH (Figures 1(A) and 1(B)). This enzyme hydrates singly substituted acyclic epoxides as well as 1,1- and *cis*-1,2-disubstituted compounds (Figure 1(C)). Among aliphatic cyclic epoxides, the optimum ring size appears to be seven. For those substrates that are metabolized by both enzymes, the sEH appears to be the more active enzyme. However, the murine sEH fails to metabolize arene oxides at an appreciable rate. Among aliphatic cyclic compounds only cyclodecane epoxide is metabolized rapidly by sEH. It is not known if this observation will hold with other species such as rabbits and humans. Both enzymes metabolize most monosubstituted epoxides (Figures 1(D)–(G)). The sEH metabolizes a variety of acyclic epoxides at a high rate, but metabolizes 1,1-disubstituted epoxides poorly. The rodent sEH metabolizes *cis*-β-substituted styrene oxides much more slowly than the *trans* isomers, which is the basis of commonly used assays to distinguish the two enzymes (Figures 1(H)–(K)). However, the human enzyme actually hydrates *cis*-stilbene oxide slightly faster than the *trans* isomer.[34]

3.18.3 ANALYTICAL METHODS FOR EH ENZYMES

3.18.3.1 General Methods

Wixtrom and Hammock[16] give an extensive analytical background for the analysis of EHs, whereas detailed methods are provided elsewhere.[35] A variety of substrates, analytical tools, and methods can be used successfully. In general, methods based on chromatographic separation and spectral identification of products should be used if a new system is being evaluated. Partition methods offer the best compromise between reliability and speed of analysis. These methods are based on the differential partitioning of the epoxide moiety vs. its diol on moderately sized compounds between water or a water–methanol or acetonitrile mixture, and a hydrophobic solvent, such as isooctane, dodecane, carbon tetrachloroide, or trichloroethane. An advantage is that the same method often can be used to monitor glutathione transferase activity,[36,37] but this also is a caution that such alkylation of nucleophiles by epoxides interferes with estimating rates of diol formation. This can be addressed by a two-step extraction procedure using hexanol or ethyl acetate to remove the diol from more polar materials. For kinetic studies, spectral substrates offer many advantages, but the spectral assays are limited in terms of specificity or sensitivity.

3.18.3.2 Assay Methods for the Microsomal Epoxide Hydrolase

Traditionally, a partition assay based on radioactive styrene oxide (Figure 1(D)) has been used to monitor mEH.[38] The assay has historical and comparative value, but it has many disadvantages relative to other substrates. Among other problems are lack of specificity of the assay because styrene oxide is a good substrate for the sEH and some glutathione transferases, and styrene oxide reacts with the sulfhydryl groups on a protein's surface. The substrate is unstable on storage, volatile, mutagenic, inhibits many enzymes including the sEH, and gives a high background. As an example of a GLC (gas–liquid chromatography) method, Brooks[39] developed an assay based on the halogenated cyclodiene HEOM (1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-methanonaphthalene). With careful selection of internal standards or care in developing column techniques that do not lead to decomposition of epoxide substrates, GLC offers a rapid method for screening large numbers of substrates.[40–42] Similar techniques can be applied to polycyclic aromatic compounds.[43] An excellent thin layer chromatography (TLC)-based assay is the hydration of radioactive benzo(*a*)pyrene 4,5-oxide (Figure 1(B)),[44] and this assay is generally applicable to a variety of radiolabeled or other substrates that can be easily detected.[35,45] Prelayer TLC plates allow reaction mixtures to be spotted directly and the new generation of scanners for TLC circumvent scraping TLC plates.[6,34] The TLC method is generally applicable. For instance, both TLC and a partition method can be used with the herbicide Tridiphane. Although this compound is turned over very slowly by the mEH, the very high stability of the molecule to chemical hydrolysis leads to an assay with a very high signal-to-noise ratio.[7] The same observations are applicable to epoxyandrost-4-en-3-one, which shows high signal to background hydrolysis with mEH.[46] One seldom needs the higher resolution of HPLC, but the technique offers quantitative detection

of a variety of substrates and is generally applicable. For instance, the glycidyl ether of 4-nitrophenol (Figure 1(F)) was used successfully for the analysis of both the mEH and sEH.[47–49] A good partition assay for the mEH is based on *cis*-stilbene oxide. This system has the advantage of using commercial substrates which are easy to radiolabel and is useful in combination with the corresponding *trans*-stilbene oxide for the sEH (Figures 1(C) and 1(I)).[35,50,51] The application of a partition assay to unlabeled molecules was demonstrated in an mEH assay based on safrole oxide.[52] The partition method can be applied to any compounds where there is a large difference in polarity between the diol and the epoxide. However, because of the large size of most arene oxides and steroid oxides, partition assays for them are laborious.[35,53–55]

Spectral assays for the mEH are very attractive. The most well-documented assay is based on phenanthrene oxide and other K-region arene oxides (Figure 1(A)).[56] For higher sensitivity, apply fluorescent techniques to these compounds.[57,58] For some primary epoxides, couple formation of the diol with reduction of NAD^+ or dyes for analysis.[59] 4-Nitrostyrene oxide was the basis for a spectral assay for the mEH[60] which shows low rates with the sEH.[61] Epoxyethoxyl derivatives of chromophores are attractive spectral substrates, but they prove too unstable in practice.[62]

3.18.3.3 Surrogate Substrates for the Soluble Epoxide Hydrolase

The most commonly used substrate for the sEH is *trans*-stilbene oxide (TSO) which can be monitored by partition, TLC, GLC, or HPLC (high-pressure liquid chromatography) (Figure 1(I)). Earlier substrates included epoxides on very large molecules, partitioned between alcohol, water, and hydrocarbons,[63] and later *trans*-β-alkyloxides, which partitioned very well. These substrates yielded a much higher rate than TSO, but were more volatile (Figure 1(H)).[64] *trans*-Benzylstyrene oxide was found to have the attributes of both classes of substrates (Figure 1(J)). It is turned over at a high rate by the sEH; in several species it appears selective for the enzyme; the partition data are excellent, whereas rates of evaporation and buffer-catalyzed hydrolysis are very low.[34] Fatty acid epoxides are excellent substrates for the sEH. The simplest in the series is *trans*-epoxystearate which can be prepared from commercially available ^3H or ^{14}C oleate (Figure 1(H)). Partition methods are poor with both the free acid and methyl ester, but both are easily monitored by TLC.[34] A variety of monosubstituted epoxides including glycidyl ethers, epoxyalkanes, and allylbenzene oxide are excellent substrates for both the mEH and sEH (Figures 1(E)–(G)).[16]

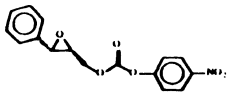
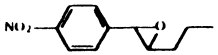
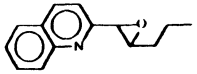
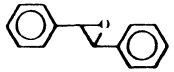
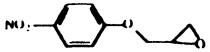
Spectral substrates clearly are in demand in the field but in crude preparations are likely to detect transferase activity as well (Table 1). A large (for the epoxide–diol) difference spectrum exists for *trans*-stilbene oxide, however, the λ_{max} is in the UV.[65] Loss of the aromatic nature of the epoxide and thus conjugation with aromatic rings probably accounts for the spectral shift with phenanthrene oxide (PO), TSO, 4-nitrostyrene oxide (PNSO), *trans*-β-propylstyrene oxide (ENPO), and similar compounds. An interesting concept was developed with *trans*-1,2-epoxy-1-(2-quinolyl)-pentane (EQU-5) where hydrogen bonding to the quinolyl nitrogen is thought to contribute to the large spectral difference between the diol and the epoxide (Figure 2).[61] This concept of hydrogen bonding was extended by Dietze *et al.* resulting in compounds in which the product diol would cyclize and rearrange to yield 4-nitrophenol. The (2*R*,3*R*)- or (2*S*,3*S*)-2,3-epoxy-3-phenylpropyl carbonates (*R* or *S*-NEPC, respectively) proved to be excellent substrates easily prepared from commercially available materials (Figure 2).[66] This concept was applied to a variety of carbonates or cinnamic acid derivatives which cyclized to release a product that could be detected colorimetrically (4-nitrophenol), fluorometrically (coumarin), as a stain (naphthol), or by coupling to another reaction (ethanol). The concept is generally applicable for a wide variety of substrates for sEH, mEH, or glutatione (GSH) transferase as well as for prodrugs activated by these enzymes.

Each of these assays offers advantages and limitations. The TSO assay is based on a commercially available product. The EQU-5 assay has the advantage of taking place in the near UV allowing the use of an inexpensive microplate reader and of being a product appearance assay. The *S*-NEPC assay is colorometric, very sensitive, uses an easily synthesized optically active substrate, but suffers from interference caused by esterases. A highly sensitive, specific, commercially available, colorimetric, product appearance assay for the sEH as well as the mEH is still lacking.

3.18.4 CATALYTIC MECHANISM

A variety of possible catalytic mechanisms for an epoxide hydrolase based on epoxide chemistry may be envisaged,[4,67] including an

Table 1 Comparison of properties of spectral substrates for soluble epoxide hydrolase.

Structure	*Abbreviation (EH)*	*Difference* λ_{max}	*Difference* ϵ ($M^{-1}cm^{-1}$)	*Product appearance assay*	*Rate (relative to TSO)*	K_m (μM)	*Interference*
	*S*-NEPC (sEH)	405	10 600	Yes	288	16	GSH transferase esterase
	ENP-5 (sEH)	302	1520	No	160	1.7	GSH transferase
	EQU-5 (sEH)	315.5 320	1500 3700	Yes No	48	25	GSH transferase
	TSO (sEH)	229	15 000	No	10	5	GSH transferase
	PNSO (mEH) (sEH)	293	1350	No	0.2		mEH GSH transferase
	ENPO-3 (mEH) (sEH)	302	1560	No	0.09		GSH transferase mEH
							

See also Chapter 19, this volume.

acid-catalyzed mechanism involving a carbocation at one epoxide carbon and resulting in racemization (Figure 3(A)), a metalloenzyme system which could result in *cis*-hydration (not shown) or *trans*-hydration with the metal acting as a Lewis acid to activate water, or a more generalized base-catalyzed mechanism resulting in *anti*- or *trans*-hydration with retention at one carbon and inversion at a second (Figure 3(B)). Examples of all of these mechanisms will possibly be found, but the mammalian microsomal and soluble enzymes simplistically fit the third situation in terms of the stereochemistry and geometry of the product. Leukotriene A_4 hydrolase appears to be a zinc metaloenzyme and will not be discussed here. The observation that the epoxide hydrolases may be in the α β-fold family of hydrolases stimulated several experiments which strongly support a mechanism involving neither a free carbocation nor metal ions. Both sEH and mEH clearly use a two-step mechanism as shown in Figure 4, rather than the single-step general base mechanism shown in Figure 3(B).[68,69]

For many years the simplest interpretation of a variety of experiments regarding the mechanisms of epoxide opening involved general base

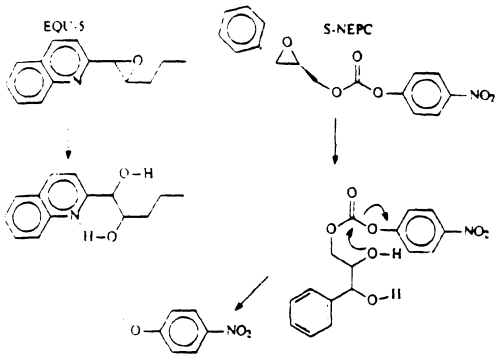


Figure 2 Two spectral substrates for analysis of sEH. The spectral change associated with EQU-5 is thought to be associated with destruction of the aromatic nature of the epoxide ring and hydrogen bonding to the quinoline nitrogen. The diol of *S*-NEPC cyclizes to release 4-nitrophenol as a yellow product.

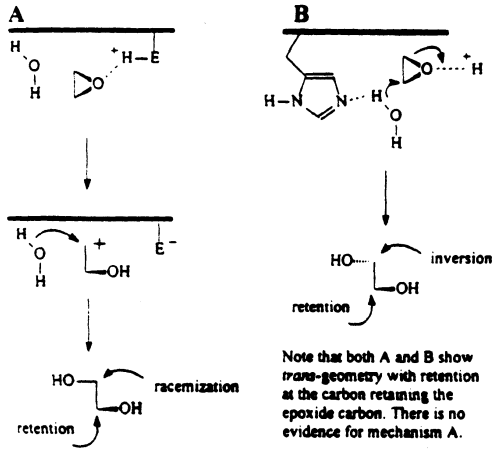


Figure 3 Proposed mechanisms for epoxide hydration showing the stereochemistry of the resulting diol. (A) In a carbocation mechanism the epoxide is activated by the addition of a proton. The lack of racemization of either diol carbon resulting from the action of either the mEH or sEH and the position of water attack argue against this mechanism. (B) A mechanism in which water is activated by a general base and attacks an epoxide directly explains the stereochemistry of hydration and has been generally accepted for many years. Information indicates that this single-step mechanism is not correct.

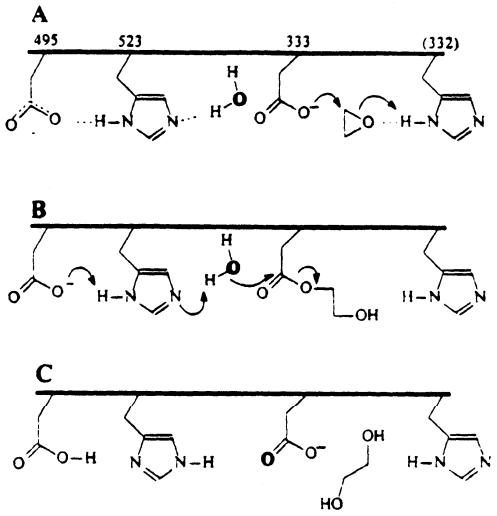


Figure 4 A two-step mechanism of epoxide hydration consistent with the evidence on catalysis of both the mEH and sEH and drawn analogously to the common drawing of the mechanism of serine esterases. The amino acids are numbered according to the sequence derived from the murine sEH. (A) The epoxide is activated by torsion and or polarization of the C—O bond shown hypothetically by proton donation from His332, and a backside nucleophilic attack occurs from Asp333. (B) This results in a covalent hydroxyacyl enzyme which can be isolated. In a second step water is activated by His523 which is in turn oriented and activated by the basic Asp495. (C) Following proton shuttles not shown, the diol is released and the nucleophilic Asp333 is regenerated. Note that the oxygen from water shown in bold is added to the nucleophilic Asp in the first turnover, not to the epoxide. Not shown are transition states in which the original acidic carbon of Asp333 approaches a tetrahedral form.

activation of water and the backside attack of this water molecule on the epoxide carbon (Figure 3(B)).[15,70,71] Numerous observations are consistent with this mechanism including the *trans* geometry of hydration involving an apparent backside attack of water on the epoxide,[12,41,72] kinetic isotope effects and Hammett correlations,[71,73,74] usually high regioselectivity with attack at the less hindered carbon,[70] enantioselectivity of hydration with retention at one carbon and inversion at the carbon attacked by water,[75,76] the incorporation of heavy water into the inverted carbon,[70,77] as well as strong suggestions for the involvement of a histidine at the active site as a general base activating water.[78-80] The observations based on apparent sequence identity among the soluble and microsomal hydrolases and other members of the α β-hydrolase fold family of enzymes, suggested a two-step mechanism analogous to the action of serine esterases and lipases (Figure 4).[81,82] Numerous experiments have generated data supporting this two-step mechanism. Single turnover studies with heavy water showed that the water incorporated into the product diol comes directly from the enzyme and only indirectly from water in the mEH.[68] This elegant experiment was repeated using the sEH.[83] In Figures 4(A)–(C) the results of such a single turnover experiment with the murine sEH are shown where the ^{18}O is indicated in bold. In the first turnover there is 100% incorporation of the ^{18}O into an amino acid (suggested to be Asp333), but no incorporation into the substrate. After several turnovers, essentially all diols will have ^{18}O incorporated from heavy water, but in each case the oxygen will be donated by the carboxylate which is labeled with two ^{18}O atoms. A substrate of sEH also was shown to bind covalently to the enzyme and have a structure consistent with an α-hydroxy ester.[84] The tryptic peptide containing this α-hydroxy acyl-enzyme shown in Figure 4(B) was later isolated and identified by electrospray mass spectrometry.[85] Heavy water was shown to incorporate into the Asp333 in this peptide only in the presence of substrate.[86] In the murine-soluble enzyme, site-directed mutagenesis supports the above mechanism by implicating Asp333 and His523 as essential amino acids since mutagenesis of these amino acids eliminates >99.9% of the catalytic activity. If Asp333 is changed to Asn, the catalytic activity and its ability to form a covalent substrate adduct increases with time as the Asn is hydrolyzed back to Asp.[85] Since Asn is chemically quite stable, this observation suggests the presence of a very active water molecule near the catalytic site.

For the proposed mechanism to occur it is necessary that there is a system to activate water to attack the α-hydroxy ester. The most common general base is histidine. Catalytic histidines characteristically have a small amino acid next to them (Gly or amino acids which bond back to themselves and are sterically small such as Ser or Asn), they are conserved, usually in a kinked region, anchored by a Cys close in the linear sequence and in about half of the cases oriented by an Asp which is close in space. The large number of conserved histidines that fit these criteria suggest that the sEH may have multiple catalytic sites or have evolved from proteins with several sites. Mutagenesis suggests that His523 is catalytic. Mutagenesis on the rat sEH yielded similar results, also implicating Asp333 and His523 as catalytic.[87] Mutagenesis of Asp495 in the similar rat enzyme also dramatically reduces catalytic activity, whereas Glu493, Asp434, and Glu433 are essentially dispensable.[87] These data suggest that Asp495 is involved in the orientation and activation of the histidine acting as a general base. A spacially adjacent Asp can increase the pK_a of His by over 2 units since the Asp often is poorly solvated in a hydrophobic pocket and thus can act itself as a very strong base with a very high affinity for its proton (pK_a 8–9). This triad of hypothetically catalytic amino acids is conserved in all EHs so far sequenced,[88] and the corresponding His in the mEH has also been shown essential for catalytic activity.[80] Although the microsomal and soluble EHs are only very distantly related, it is likely that the major amino acids involved in catalysis will be the same in both enzyme groups since similar catalytic triads are conserved throughout the α β family.[88,89]

A variety of lines of evidence suggest that both the sEH and mEH of animals and plants act in a two-step mechanism in which a nucleophilic aspartic acid attacks the epoxide by a backside or A2 mechanism (Figure 4(A)). In a second step this α-hydroxyester intermediate is in turn attacked by a water molecule activated by a histidine–acid pair (Figure 4(B)) to release the product diol and regenerate the enzyme (Figure 4(C)). This is an analogous mechanism to that of serine esterases such as acetylcholinesterase; however, note that in the case of the EHs the acyl portion of the α-hydroxy acyl-enzyme is contributed by an aspartic acid.

Both the mEH and the sEH are in the α β-hydrolase fold family. The α β-fold family of enzymes are highly divergent in sequence and catalytic activity, but they retain a similar mechanism and structure. Each enzyme in the family has an α β sheet consisting of eight β sheets joined by α helices. This core holds

the catalytic triad in an arrangement which represents the mirror image of the triad of serine proteases described elegantly by Blow *et al.*[90] In the EHs the catalytic nucleophile is near the center of the molecule with the remainder of the catalytic triad extending toward the C terminus.

3.18.5 INHIBITION

Only weak inhibitors are known for the microsomal EH. The best inhibitors of this enzyme are simply the alternate substrates trichloropropene oxide and cyclohexane oxide, which have been known since the beginning of the 1970s.[12,45,91] Both inhibitors enhance the effect of a variety of epoxide-containing xenobiotics by selective inhibition of the mEH. They have little effect on the sEH and are of moderate water solubility; however, they are volatile. At concentrations that will cause significant inhibition of mEH, trichloropropene oxide will kill many cells and denature some proteins.[92] Both inhibitors are valuable tools; however, they must be used with caution when implicating mEH in a metabolic pathway. For instance, inhibitory potency of a single compound will vary with many factors including incubation conditions, enzyme concentration, and the K_m of the substrate used. Trichloropropene oxide is especially sensitive to conditions of its use.

Of the two compounds, cyclohexane oxide is the weaker but more selective inhibitor of the mEH. It is not known what the detailed mechanism of the inhibition is and neither has there been extensive structure optimization within this series of compounds. Examination of a series of epoxide-containing cycloalkanes revealed that the cyclohexane derivative was a potent inhibitor (I_{50} of 4×10^{-6} M) and only the cyclodecane derivative was within an order of magnitude of this potency. Turnover of cyclopentane and cyclohexane oxides was very slow with the mEH. The rate of turnover of cycloheptane oxide was the fastest in the series, but epoxides on all larger ring systems were turned over much faster than cyclohexane oxide.[93]

Trichloropropene oxide (TCPO) is the most commonly used EH inhibitor. It can be a quite potent inhibitor with purified or enriched preparations of mEH, but in the presence of glutathione transferases, free sulfhydryls, and other proteins, its potency drops off dramatically. There has been a systematic study of this and related compounds[94] where it was found that trichloropropene oxide was an alternate substrate for the mEH which was quickly turned over by the enzyme. Interestingly, some glycidyl ethers were found to be mEH inhibitors on both mammalian and insect mEHs but they are rarely used.[39,95]

Much better inhibitors are known for the soluble epoxide hydrolase. The most potent compounds described are the chalcone oxides with 4-phenyl chalcone oxide giving an I_{50} of 6×10^{-8} M. The corresponding 4′-phenyl derivative was an order of magnitude less active. The 4-fluorochalcone oxide is about 40-times less active than the 4-phenyl, but it is often used as a compromise between solubility and potency. The compounds are slowly reversible inhibitors which show slow tight binding kinetics.[96] These kinetics often are associated with transition state mimics,[97] but the data are consistent with the chalcone oxides being substrates which are turned over slowly.[98] When the enantiomers of 4-fluorochalcone oxide were examined with the murine sEH, the potency was similar, indicating a lack of enantiofacial selectivity in this compound.[99] The chalcone oxides show little inhibition of mEH or GSH transferase making them very useful and selective compounds. However, the unsubstituted chalcone itself is quite a potent inhibitor of some GSH transferase isozymes while not inhibiting either mEH or sEH.[100]

A related series of compounds, the *trans*-phenylglycidols, proved to be potent, slow binding inhibitors with high stereospecificity. The most potent inhibitor of this series was (*S,S*)-2,3-epoxy-3-(4-nitrophenyl)glycidol. The potency of the *S,S* isomer varied from over 540 times greater than the *R,R* enantiomer for the human sEH to over 980 times for the rabbit sEH.[101,102] If the free hydroxy group was blocked, the potency, slow binding character, and stereoselectivity was lost. Other members of a limited series showed variable potency as inhibitors with stereospecificity dropping with potency.[99,101] Both the glycidols and chalcone oxides are thought to be substrates that are turned over slowly because the polar carbonyl or alcohol displaces the active water from the enzyme and/or by stabilizing the acyl enzyme intermediate. Neither the existing glycidols nor the chalcone oxides represent the irreversible inhibitors which are the most valuable for probing biological roles. A variety of other compounds are moderate inhibitors of the EHs including drug metabolites.[103]

3.18.6 TISSUE DISTRIBUTION

Epoxide hydrolases have been found in nearly all tissues for which measurements have been made. The relative levels between species for a given tissue, or between tissues in a

given species, and even among individuals for a given tissue, can vary by as much as 1000-fold. In a sample of over 100 human livers, the level of mEH varied by 63-fold and the level of sEH by 539-fold.[104] This tremendous variability is one of the more fascinating attributes of these enzymes, and may provide us with clues to help determine their *in vivo* function.

3.18.6.1 Microsomal Epoxide Hydrolase

3.18.6.1.1 Tissue and animal expression

Microsomal epoxide hydrolase appears to be ubiquitous in mammalian tissues having been found in at least 26 different rat organs and tissues.[105] Most of the work with these enzymes has been done using rats, mice, and human liver, but mEH also has been found in the adrenal glands of humans,[106] in monkeys,[36,107] in rabbits,[107-109] in dogs,[110] mink,[111] livestock species,[112] and fish.[113] Activities are generally highest in the liver, followed by testis, lung, and heart, all of which have similar levels of activity.[114] In human lungs, mEH was not correlated with the level of tobacco use in lifelong or recent smokers.[115] This is rather surprising given that cigarette smoke is known to contain a variety of polyaromatic hydrocarbons some of which are known to induce mEH in the liver. The mEH also has been measured in various components of human blood.[116] Most of the activity was found in the lymphocytes (75–80%) with much lower activity in the monocytes (20–25%), granulocytes (0–8%), or platelets (0–10%), and undetectable activity in the erythrocytes and plasma. The interindividual variation was approximately twofold in 27 subjects, while the intraindividual variation was approximately 10% over the course of 18 days.

Honscha *et al.*[117] provided evidence in the rat that alternate splicing of two distinct first or 5′ exons occurs in a tissue-specific manner and that these alternate mRNAs are differentially inducible. The resultant proteins, however, are identical since the differences between the two exons occur upstream from the ATG start site. They propose that the differential inducibility occurs as a result of at least two different promoters. This would provide an explanation for the tissue specific differences in expression of mEH.

3.18.6.1.2 Cancer cells

Another important area of research is the expression of epoxide hydrolases in transformed cells.

Ding *et al.*[118] measured the steady-state levels of mEH mRNA in persistent hepatocyte nodules and hepatomas in rats induced by the Solt–Farber chemical carcinogenesis procedure. EH mRNA was increased 14-fold in nodules compared to the level in normal liver. Phenobarbital treatment of rats which had liver nodules led to a superinduction in mEH mRNA in the nodules as well as in the tissue surrounding the nodules. Hypomethylation of the mEH gene is correlated with increased expression of mEH steady-state levels of mEH mRNA.

Kondo *et al.*[119] measured the steady-state levels of mEH in rat liver following a single initiating dose of diethylnitrosamine. mEH mRNA was found to be induced by more than 10-fold and became constitutively expressed during the process of hepatocarcinogensis. There was no change in the initiation start site between induced and uninduced rat livers. The authors hypothesized that the altered expression of mEH was the result of changes in cell regulation associated with altered cell growth.

The expression of mEH in neoplastic tissues in humans has been studied in colon tumors,[120] lung tumors,[121] head and neck squamous cell carcinoma,[122] breast tissue,[123,124] liver,[125] the adrenal gland,[126] and in kidneys.[127] Unfortunately, there is no consistent pattern of expression for mEH in these cells that could be exploited for a general strategy for prodrug design. mEH was not expressed in either mouse colon adenocarcinoma Co38 or normal mouse colon tissues, whereas it was expressed in human colon peritumoral and tumoral tissues at similar levels.[120] This suggests that in terms of mEH expression, the mouse colon adenocarcinoma Co38 model may not be a good model to predict epoxide metabolism in humans. In lungs, mEH protein level was significantly decreased in tumors compared to nontumoral tissue.[121] In human head and neck squamous cell carcinoma, mEH expression was lower in tumors compared to corresponding nontumoral tissues.[122] Breast tumors were characterized by consistent expression (89% of the patients) of mEH. Adjacent non-neoplastic breast tissue also demonstrated mEH immunoreactivity but was less intense than tumor cells. mEH was not expressed in stromal cells, mast cells, or inflammatory cells present either surrounding the tumor of within non-neoplastic breast epithelium. In another study, several drug-metabolizing enzyme systems were evaluated in both breast tumors and their corresponding peritumoral tissues in 12 patients. mEH was expressed at similar levels in tumors and peritumoral tissues.[123]

Human hepatocellular carcinomas were characterized by a consistently high expression of mEH as determined by immunohistochemistry[125] of 31 human liver biopsies. The level of mEH in liver lesions was equal to or more than that found in normal liver tissue. In tumors, immunoreactivity for mEH was found in tumor cells but not in bile ducts, inflammatory cells, or blood vessels. mEH activity was not correlated with cytochrome P450 or GST activity.

The level of mEH activity in normal human adrenal tissue and corresponding hyperplasia, adenomas, and carcinomas was measured[126] and found to be reduced in adrenocortical carcinomas. The level of mEH in slightly atrophic adrenal cortex surrounding adrenocortical carcinomas was also found to be reduced.

The cellular distribution of mEH in normal and neoplastic human kidneys was found to be widely distributed.[127] The main localization of mEH immunoreactivity in normal human kidney tissue was in the proximal and distal tubule epithelial cells. It was also identified in the epithelium of the collecting ducts, in vascular endothelial cells, and in cells lining glomerular capillaries. Positive immunoreactivity was found in all renal tumors with the precise pattern of immunoreactivity depending on the cytology of the tumor cells.

3.18.6.1.3 Expression in cells and cell lines

Glatt *et al.*[128] measured the levels of mEH in 18 cell lines that are widely used for short-term mutagenicity and carcinogenicity tests. The mEH activity in these cell lines displayed a wide range of activities similar to the wide range of activity reported for various tissues. Cell lines cultured originally from rat liver exhibited enzyme activity levels that were drastically different from freshly isolated rat hepatocytes, suggesting that cell-based assays are no better than and are probably less predictive of metabolic pathways than are rodent studies for predicting metabolic pathways in humans. In a study of mEH activity in oval cells, Steinberg *et al.*[129] found that freshly isolated oval cells and an established oval cell line had approximately identical mEH activities. Liver cells immortalized using the SV40 T antigen expressed mEH activity at levels approximately equal to the levels found in liver tissue.[130] These cell lines also expressed other phase II enzymes and phase I enzymes equal to the levels found in liver tissue and may prove to be useful for toxicological studies.

3.18.6.2 Soluble Epoxide Hydrolase

3.18.6.2.1 Tissue and animal expression

Like mEH, sEH has been found in all tissues and animals for which measurements have been taken. In many studies the activities of both enzymes have been measured using the same tissue after fractionation into cytosolic and microsomal components. A consistent pattern that has emerged from these studies is that expression of mEH and sEH is completely independent. Since sEH was discovered several years after mEH, not as many tissues or different animals have been assayed.[36,111–113] One striking finding is that the level of sEH activity in mouse liver is 100-fold greater than in rat liver in spite of the similarities of their respective cDNAs. Rats have highest activity in the kidneys, followed by heart, liver, brain, lung, testis, and spleen.[131,132] In mice most of the activity is in the liver followed by the kidney, breast tissue, and heart, with much lower levels in the lung and testis.[114,133–135] Work in humans has included measurements in the various components of the blood. Lymphocytes contained virtually all of the sEH activity and had a surprisingly high temperature optimum of 60°C.[116] sEH was also measured in adult and fetal tissues with adult tissues generally showing higher levels of activity than fetal tissues.[136] One exception to this was that fetal lung tissue had approximately five times the specific activity of adult lung tissue. This low activity in adult lung tissue may be of toxicological significance because it has been shown that cigarette smoke inhibits sEH.[115] Significant levels of both mEH and sEH occur in the skin.[137,138]

3.18.6.2.2 Cancer cells

Few studies have investigated the activity of sEH expression in neoplastic tissue or in isolated cancer cells. In an early study,[36] there was no detectable difference between the activity of sEH in control and neoplastic human liver. However, sEH was lower in adrenal tumor cells compared to the normal surrounding tissue.[126]

3.18.6.2.3 Expression in cells and cell lines

The levels of sEH in established cell lines is generally very low. For example, the 18 cell lines that were mentioned above for analysis of mEH expression were also used to measure sEH activity. The results showed that in most

cases, the activity was not measurable and was very low in the others.[128] sEH was also very low in hybrid cell lines established by fusion of liver parenchymal cells from adult rat with cells from a Reuber hepatoma cell line[139] and in hepatoma cells.[140]

The importance of sEH in protecting cells from genetic and cytotoxic damage due to epoxide exposure has been debated for many years. Most of the experimental evidence suggests that sEH can protect cells from epoxide toxicity. For example, sister chromatid exchange in human lymphocytes exposed to *trans*-β-ethylstyrene-7,8-oxide was shown to be potentiated by 4-fluorochalcone oxide, an inhibitor of sEH.[141] In another study, a series of *trans*-β-substituted styrene 7,8-oxides was used to measure mutation frequency in the *Salmonella typhimurium* reversion assay or at the *hprt* locus in V79 cells, and sister chromosome exchanges in human lymphocytes and V79 cells. None of the compounds caused mutations as measured by either assay, whereas sister chromatid exchanges were induced in sEH-deficient V79 cells as well as sEH-proficient human lymphocytes.[142] The results of these studies suggest that in some cases sEH can protect cells from mutagenicity.

3.18.7 MOLECULAR BIOLOGY

3.18.7.1 Microsomal Epoxide Hydrolase

3.18.7.1.1 Protein purification and analysis

It is now well established that sEH and mEH are distinct enzymes[36,143,144] with the activity in the cytosolic fraction not being due to contamination from the microsomal fraction. Classical purification techniques have been reviewed by Lu and Miwa.[15] In addition, most of the available data from rodent and human studies suggests that there exists only one enzyme in each of these two classes. Work at the protein level has shown that mEH activity in rats that was initially thought to be due to several isozymes[145,146] is due to a single isozyme having different phospholipid content.[147] In human liver there also appears to be only one isozyme.[148]

Work at the protein level has led to some interesting results regarding the membrane topology and *in vivo* function of mEH. Previous work suggested that mEH contained six transmembrane domains and was therefore imbedded in the microsomal membrane.[149] More recent work suggests that mEH is retained in the microsomal membranes by a single 20-residue transmembrane segment located at the N-terminus, and the majority of the protein is exposed at the cytosolic membrane surface.[150] This hypothesis was further supported by work showing that the N-terminal 20 amino acids of mEH could be replaced by a cytochrome P450 anchor signal sequence. The resultant fusion protein was co-translationally inserted into dog pancreas microsomes in a cell-free translation system, whereas a mutant mEH lacking these 20 amino acids was not cotranslationally inserted.[151] This work presents a rather interesting picture of the mEH as being largely a cytosolic protein. Alves *et al.*[152] have reported that the mEH can transport bile acids such as taurocholate with a K_m of 350 μM and a V_{max} of 30 nmol min^{-1} (mg protein)$^{-1}$. Although this is a rather high K_m, these results suggest a unique hypothesis for an *in vivo* function of mEH. The orientation of mEH may provide some insight into its release from the endoplasmic reticulum and its appearance in both liver cytosol and blood. In this case, mEH is termed the preneoplastic antigen and has been proposed as a biomarker for liver cancer and cell damage.[153,154]

3.18.7.1.2 cDNA cloning and heterologous expression

cDNA clones for rat,[149] human,[155] and rabbit[109] mEH have been produced and isolated. Their sequences are >75% similar and their hydrophobicity plots are also very similar.[109] All have 455 amino acid residues with a strongly hydrophobic 20-amino acid region at the extreme N-terminus end of the protein. This region is the presumed anchor region mentioned above. The functional equivalence of these cDNAs to the native enzyme has been assessed by heterologous expression in a variety of systems including COS-1 cells,[155] yeast cells,[156] and in the baculovirus system.[157]

The role of mEH in protecting cells from mutagenicity and cytotoxicity has been indirectly established by a number of studies. The advent of cDNA cloning and cell transfection have now made direct experimental comparisons possible. There have been two systems of this type used for the expression of human mEH *in vitro*: a yeast-based system and mammalian cell expression systems. The yeast-based system was used to study the metabolism of carbamazepine epoxide[158] and benzo(*a*)pyrene.[159]

A yeast-based expression system was also used to coexpress human mEH and cytochrome P450 1A1 in the same cell.[159] This offers the advantage of more closely mimicking the *in vivo* situation and their results closely matched

previous results with rodent microsomes. However, yeast-based systems are less useful for defining mechanisms of toxicity since the host cells are not analogous to mammalian cells and extrapolation then becomes subjective. An approach to lessen this concern has been used by several workers for the expression of mEH in mammalian cells in order to determine its role in drug and xenobiotic metabolism.[160-166] All of these studies used an expression system based on the lymphoblastoid cell line.[162] However, none specifically addressed the role of mEH in protecting cells from mutagenic or cytotoxic epoxides. One study addressed this issue by expressing the rat mEH in BHK21 Syrian hamster cells and measuring the ability of the enzyme to protect cells from the mutagenicity and cytotoxicity of benzo(*a*)pyrene 4,5-oxide and *anti*-7-methylbenz(*a*)anthracene-3,4-diol 1,2-oxide.[167] Despite the ability of the recombinant cells to metabolize benzo(*a*)-pyrene 4,5-oxide by the high level of endogenous GSH transferase, mEH was found to afford added protection to the cells. mEH had no effect with the *anti*-7-methylbenz(*a*)anthracene-3,4-diol 1,2-oxide, which was expected because this compound is not a good substrate for mEH.[168]

3.18.7.1.3 Gene mapping and cloning

Work using the rabbit,[109] human,[155] and rat cDNAs[169] as probes on Southern blots suggest that mEH exists as a single gene.

The mEH gene is on chromosome 1p11-qter in humans and chromosome 1 in mice.[155,170] Based on homology mapping in the mouse, the human gene is most likely on the distal region (i.e., approximately 1q41) of chromosome 1. As far as is known, no genetically based diseases that are easily correlated with the expression of mEH are located in this area of the human map. Genomic clones of both the rat and human mEH have been characterized by partial or complete sequence analysis, respectively.[169,171] Regions that regulate basal transcription of the rat mEH gene have been identified 5′ to the gene.[172-174] With the exception of a region that includes the enhancer region of the rat mEH gene, none of these DNA sequences are present in the human mEH gene.[171]

3.18.7.1.4 Gene polymorphisms

It has been known for some time that variation in the level of mEH activity occurs in human liver.[104] Variation in mEH activity at the tissue level could be due to tissue specific promoters influencing the levels of mEH message or regulation of mEH mRNA or protein stability. In addition, polymorphism at the mEH locus could explain differences in mEH specific activity. As described earlier, one clinical manifestation of mEH variation is thought to occur in the development of fetal hydantoin syndrome.[175] To directly test the hypothesis that a genetic defect in mEH function could be responsible for drug toxicity, Gaedigk *et al.*[176] compared mEH gene sequence data in patients with anticonvulsant adverse reactions and in control subjects in which no *in vivo* or *in vitro* toxicity to anticonvulsants could be demonstrated. Although many of the mutations found in these patients were the same ones found by Hassett *et al.*,[177] they found no correlation between any specific mutation and drug susceptibility. They concluded that a genetic defect in the structure of the mEH gene was unlikely to be responsible for predisposing patients to anticonvulsant adverse reactions.

3.18.7.1.5 Regulation of expression (stimulators and inducers)

In vitro mEH activity can be stimulated by numerous chemicals including heterocyclic amines (harman),[178] ketones (benzil),[179] alcohols (cyclohexanol), metyrapone,[12,180] flavones,[181] and chalcone oxide.[182] The extent of stimulation can vary with the substrate used.[91] Some of these stimulators are believed to act by increasing substrate solubility and subsequently substrate accessibility to the enzyme.[13] However, strict structural requirements[178,180,181] and noncompetitive stimulation kinetics increasing both apparent V_{max} and K_m are consistent with an allosteric binding site.[179] One hypothesis suggests allosteric binding reduces substrate inhibition at high concentrations of substrate.[183] These stimulators of enzyme activity are an invaluable tool for deducing the role of mEH during *in vitro* metabolism experiments.[183,184] The existence and effects of endogenous mEH stimulators are as yet unclear.

Activity of mEH can be induced by numerous compounds including classical cytochrome P450 inducers (phenobarbital, 3-methylcolanthrene (3-MC), 2-acetylaminofluorene (2-AAF), and polychlorinated biphenyls),[17,143,185] imidazoles,[186,187] organosulfurs,[188,189] steroids,[18,174] heavy metals,[190] and peroxisome proliferators.[191-195] Each type of inducer can have variable effects upon expression of mEH activity within different tissues, sexes, strains, and species. For instance, 3-MC fails to induce

liver mEH activity in female but not male rats or either sex of mice,[196] whereas 2-AAF produces an effect only in rat and not mouse liver.[197] Generally, induction tends to be greatest in the liver[134,198] but notable exceptions exist.[190] An extensive body of literature exists regarding inducers of mEH activity.

Both transcriptional activation and post-transcriptional regulatory mechanisms have been implicated in the induction of mEH expression. Transcriptional activators account for the majority of known induction schemes. Of these, most have characterized increases in the mEH mRNA synthesis rates in rodent liver with inducers such as imidazole,[186] phenobarbital,[199] and diethylnitrosamine.[119] However, transcription rates are sensitive to xenobiotic induction on a tissue-selective basis. For instance, nuclear run-off experiments have shown that lead acetate increases mEH gene transcription rates in kidney but not liver.[190] Metallothionein is believed to play a role in this activation of transcription; however, consensus metal response elements (MREs) in the 5′ flanking region of the rat mEH gene are notably absent.[169] Single-dose diethylnitrosamine (DEN) administration to rats produces concomitant increases in mEH transcription rates, protein, and activity in liver.[200,201] Likewise, phenobarbital produces a rapid and transient increase in mEH transcription rates.[199] However, persistent hepatocyte nodules induced by DEN contain 14-fold the steady-state mRNA levels and hypomethylation of CCGG sites of the mEH gene relative to normal surrounding tissue.[118] In contrast, phenobarbital induced steady-state message in these nodules without hypomethylation of the gene. Although a causal relationship between gene methylation and induction has yet to be established, these results illustrate the existence of multiple pathways by which xenobiotics can stimulate mEH gene transcription. Another induction scheme has been proposed for DEN whereby this potent carcinogen appears to reduce one of the binding factors of a bipartite suppressor in 5′ regulatory sequences of the rat mEH gene.[173] Also, a region of the mEH promoter represses mEH transcription by synthetic glucocorticoids in a manner dependent upon the glucocorticoid receptor but apparently independent of the corresponding response element.[202] In summary, the multiplicity of regulatory elements and inducers indicate that additional effort will need to be made to decipher transcriptional control of the mEH gene.

Post-transcriptional regulation is another mechanism by which xenobiotics have been shown to alter mEH gene expression. For instance, pregnenalone-16α-carbonitrile stabilizes mEH mRNA as shown by decreased transcription rates and nuclear pre-mRNA levels but increased polyribosomal message.[202] Also, mEH message stabilization appears to account for the age-dependent increase of pre-mRNA message in male rats that cannot be explained by increased transcription rates.[203] Alternate splicing of message has also been described as a potential regulatory mechanism for the mEH gene.[117] Two of three different mRNAs derived from the single mEH gene have been cloned and contain identical peptide coding regions but have different first exons. In addition, only one of these mRNAs is inducible by Aroclor 1254. Additional research is necessary to determine if these first exons regulate inducibility by altering message stability or whether there are distinct control regions in the parent gene for each message. Other mechanisms of post-transcriptional control of mEH activity, such as phosphorylation and protein stability, invite further investigation.

3.18.7.2 Soluble Epoxide Hydrolase

3.18.7.2.1 cDNA cloning and heterologous expression

The sEH is easily purified in a single step by hydrophobic binding to benzylthio-sepharose followed by selective elution with 4-fluorochalcone oxide.[204,205] The purified protein was used to generate peptide sequence information and antibodies, ultimately resulting in its cloning. The first report of a cDNA for soluble epoxide hydrolase was provided by Arand *et al.*[206] in which the sequence of a 680 bp clone was reported for rat sEH. This clone included the 3′ end of the protein and contained a Ser-Lys-Ile carboxy terminus, which is highly homologous to the Ser-Lys-Leu carboxy terminus found in many peroxisomal proteins. The authors speculated that the slight difference was sufficient to convert the signal sequence into an impaired and therefore ambivalent targeting sequence directing the sEH to both peroxisomes and cytosol. The implications of sEH targeting have been reviewed.[207]

The full-length cDNAs for the rat,[208] mouse,[209] human,[210] potato,[7] and cress[8] sEHs have now been cloned and characterized. All of these have been expressed in heterologous systems and shown to produce proteins with the predicted physical and chemical properties.

3.18.7.2.2 Gene polymorphism, mapping, and cloning

As mentioned in the beginning of this section, the interindividual variation of sEH

in humans was 539-fold among 135 subjects.[104] An additional study in which sEH activity was measured in lymphocytes from monozygotic and dizygotic twins[211] found that control of sEH expression in humans was predominantly genetic. The study did not discriminate between a monogenic or polygenic source of variation. In addition, several nongenetic factors including fever, the menstrual cycle, and fasting influenced the expression of sEH.

As a first step to further determine the genetic basis for sEH expression, the sEH gene was mapped in both the mouse[212] and humans.[213] There was no evidence for more than one sEH gene in the mouse. The mouse mapping results showed that sEH is on chromosome 14 at band D. This region of the mouse genome is homologous to human chromosomes 8, 13, and 14. The human mapping studies confirmed these results by locating one human sEH gene on chromosome 8p21-p12. Again, no obvious connections can be made between sEH and diseases that map to this region of human chromosome 8.

3.18.7.2.3 Regulation of expression (inducers)

Being able to alter levels of sEH activity *in vivo* provides insight not only for the role of this enzyme in xenobiotic metabolism but also helps dissect the mechanisms by which expression is regulated. With the exception of ferric ions,[214] stimulators of *in vitro* enzyme activity have yet to be discovered. However, pretreatment with a wide variety of chemicals, all of which are peroxisome proliferators, induces sEH activity in rodents.[131,143,192,194,215-218] Peroxisome proliferators (PPs) are a structurally diverse group of chemicals that produce a complex but conserved physiological response characterized by enzyme induction especially of fatty acid oxidizing and or peroxisomally localized enzymes, increased size and number of peroxisomes, hepatomegaly, and hepatocarcinoma in rodents.[219-223] One hypothesis suggests that sEH may play a direct role in mitigating the hepatocarcinogenic effect of PPs in rodents.[224] This hypothesis supposes that overproduction of hydrogen peroxide associated with dramatic acyl-CoA oxidase induction[225] might result in macromolecule and membrane lipid oxidation.[226] In turn, oxidation of lipids may produce lipid epoxides[227,228] which could be subsequently detoxified by sEH *in vivo*.

Peroxisome proliferator induction of sEH activity tends to be modest in rodents. PPs that produce a weak physiological response, such as diethylhexylphthalate, induce sEH activity in mouse liver cytosol twofold or less.[143] More potent PPs, such as clofibrate, induce activity up to fourfold in the same tissues.[143,229] In addition, such induction parallels the tissue specificity of the response of mice to PPs. The greatest increase in sEH activity occurs in liver, followed by kidney, with little or no effect observed in lung, mammary gland, or testes.[135,230-232] Among several mouse strains surveyed, clofibrate treatment increased sEH activity 1.4- to fourfold with a greater effect tending to occur in strains with lower constitutive activity.[191,229] Although direct comparative studies between rodent species are lacking, rats appear to be significantly more responsive than mice to fibrate induction despite having a small fraction of the basal sEH activity.[233] Perhaps such a difference can be exploited to help understand the role of sEH induction during peroxisome proliferation. Induction studies of sEH activity in other mammalian species have yet to be performed. Failure to produce peroxisome proliferation in marmoset with long-term clofibrate administration[234] suggests that primate sEH may be refractive to induction by PPs.

Numerous investigations have addressed whether PPs preferentially alter abundance of sEH in cytosol vs. peroxisomes. In comparisons of liver subcellular fractions of control and clofibrate-fed mice, greater induction of cytosolic sEH activity has been reported.[191,216,235] However, preferential lysis of peroxisomes from treated animals during liver homogenization could produce identical results. Peroxisomal membrane integrity appears to be reduced as a result of peroxisome proliferation.[236] Gentle digitonin permeabilization of mouse liver preceding fractionation failed to show differential induction of cytosolic or peroxisomal pools of sEH activity.[235] Reduced immunochemical staining of peroxisomal sEH protein in clofibrate-treated mice may reflect disproportionate induction of other peroxisomal proteins relative to sEH.[237] Failure to show differences in induction suggests that both subcellular pools of sEH arise from the same or similarly regulated genes.

Limited work has addressed the mechanism of peroxisome proliferator induction of sEH activity. As previously mentioned, the induced enzyme has been demonstrated to be the same protein as the constitutively expressed form in mice.[98,238,239] *In vitro* translation experiments revealed an approximate doubling of sEH translation rates relative to total protein in soluble polyribosomes isolated from clofibrate treated mouse liver.[240] Further work has demonstrated a coordinated increase in cytosolic TSO hydrolase activity, cytosolic sEH protein, and mRNA in livers of three mouse

strains treated with clofibrate relative to control mice.[212] Such results indicate that the primary effect of PPs on sEH expression is to increase either transcription rates or message stability. Discovery of peroxisome proliferator response elements (PPREs) in the 5′ flanking regions of numerous other genes induced by PPs[169,223,241,242] also suggests that sEH induction occurs at the level of transcription. Such transcriptional control awaits confirmation by nuclear run-off experiments. Determination of whether transcription rates of the sEH gene are governed by a 5′ flanking PPRE or by another indirect signal transduction pathway as proposed by Meijer and DePierre[243] awaits cloning of the sEH gene and corresponding regulatory sequences.

In addition to PPs, sex hormones appear to play a role in regulating sEH expression. Several strains of mice exhibit higher liver sEH activity in males.[133,216,244,245] This sexual dimorphism begins near puberty and can be prevented by castration of males shortly after birth.[245] Studies in mice by Pinot *et al.*[246] demonstrate that castration followed by supplemental testosterone restores sEH activity to near normal male levels and has additive effects in both castrated males and females when coadministered with clofibrate. However, unlike clofibrate, testosterone induces sEH mRNA and activity in kidney more than liver and does not induce normal male mice. Such circumstantial evidence implies that clofibrate and testosterone regulate sEH expression by different but not necessarily independent pathways.

ACKNOWLEDGMENTS

This work was supported by NIEHS grants ES02710 and ES05707 and E.P.A. CR 819658–010.

3.18.8 REFERENCES

1. T. Watabe, M. Kanai, M. Isobe *et al.*, 'The hepatic microsomal biotransformation of δ5-steroids to 5α, 5βx- glycols via α- and β-epoxides.' *J. Biol. Chem.*, 1981, **256**, 2900–2907.
2. L. Iversen, V. A. Ziboh, T. Shimizu *et al.*, 'Identification and subcellular localization of leukotriene A4-hydrolase activity in human epidermis.' *J. Dermatol. Sci.*, 1994, **7**, 191–201.
3. C. R. Pace-Asciak and W. S. Lee, 'Purification of hepoxilin epoxide hydrolase from rat liver.' *J. Biol. Chem.*, 1989, **264**, 9310–9313.
4. R. E. Parker and N. S. Isaacs, 'Mechanisms of epoxide reactions.' *Chem. Rev.*, 1959, **59**, 737.
5. G. T. Brooks, 'Epoxide hydratase as a modifier of biotransformation and biological activity.' *Gen. Pharmacol.*, 1977, **8**, 221–226.
6. J. Magdalou and B. D. Hammock, 'Metabolism of tridiphane (2-(3,5-dichlorophenyl)-2(2,2,2-trichloroethyl) oxirane) by hepatic epoxide hydrolases and GSH Stransferases in mouse.' *Toxicol. Appl. Pharmacol.*, 1987, **91**, 439–449.
7. A. Stapleton, J. K. Beetham, F. Pinot *et al.*, 'Cloning and expression of soluble epoxide hydrolase from potato.' *Plant J.*, 1994, **6**, 251–258.
8. T. Kiyosue, J. K. Beetham, F. Pinot *et al.*, 'Characterization of an *Arabidopsis* cDNA for a soluble epoxide hydrolase gene that is inducible by auxin and water stress.' *Plant J.*, 1994, **6**, 259–269.
9. E. Blee, U. Stahl, F. Schuber *et al.*, 'Regio- and stereoselectivity of cytochrome P450 and peroxygenase-dependent formation of *cis*-12,13-epoxy-9(*Z*)-octadecenoic acid (vernolic acid) in *Euphorbia lagascae*.' *Biochem. Biophys. Res. Commun.*, 1993, **197**, 778–784.
10. B. D. Hammock, in 'Comprehensive Insect Physiology, Biochemistry, and Pharmacology,' eds. G. A. Kerkut and L. I. Gilbert, Pergamon, New York, 1985, pp. 431–472.
11. C. A. Mullin and B. A. Croft, '*trans*-Epoxide hydrolase: a key indicator enzyme for herbivory in arthropods.' *Experientia*, 1984, **40**, 176.
12. F. Oesch, 'Mammalian epoxide hydrases: inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds.' *Xenobiotica*, 1973, **3**, 305–340.
13. B. D. Hammock, S. S. Gill, S. M. Mumby *et al.*, in 'Molecular Basis of Environmental Toxicity,' ed. R. S. Bhatnagar, Ann Arbor Science, Ann Arbor, MI, 1980, pp. 229–272.
14. F. P. Guengerich, in 'Reviews in Biochemical Toxicology, Volume 4,' eds. E. Hodgson, J. Bend and R. Philpot, Elsevier Biomedical, New York, 1983, pp. 5–30.
15. A. Lu and G. T. Miwa, 'Molecular properties and biological functions of microsomal epoxide hydrase.' *Annu. Rev. Pharmacol. Toxicol.*, 1980, **20**, 513–531.
16. R. N. Wixtrom and B. D. Hammock, in 'Biochemical Pharamcology and Toxicology, Vol. 1: Methodological Aspects of Drug Metabolizing Enzymes,' eds. D. Zakim and D. A. Vessey, Wiley, New York, 1985, pp. 1–93.
17. U. Vogel-Bindel, P. Bentley and F. Oesch, 'Endogenous role of microsomal epoxide hydrolase. Ontogenesis, induction, inhibition, tissue distribution, immunological behaviour and purification of microsomal epoxide hydrolase with 16α,17α-epoxyandrostene-3-one as substrate.' *Eur. J. Biochem.*, 1982, **126**, 425–431.
18. F. Fandrich, B. Degiuli, B. U. Vogel *et al.*, 'Induction of rat liver microsomal epoxide hydrolase by its endogenous substrate 16 alpha, 17 alpha-epoxyestra-1,3,5-trien-3-ol.' *Xenobiotica*, 1995, **25**, 239–244.
19. S. S. Gill and B. D. Hammock, 'Hydration of *cis*- and *trans*-epoxymethyl stearates by the cytosolic epoxide hydrase of mouse liver.' *Biochem. Biophys. Res. Commun.*, 1979, **89**, 965–971.
20. N. Chacos, J. Capdevila, J. R. Falck *et al.*, 'The reaction of arachidonic acid epoxides (epoxyeicosatrienoic acids) with a cytosolic epoxide hydrolase.' *Arch. Biochem. Biophys.*, 1983, **223**, 639–648.
21. J. Nourooz-Zadeh, T. Uematsu, B. Borhan *et al.*, 'Characterization of the cytosolic epoxide hydrolase-catalyzed hydration products from 9,10:12,13-diepoxy stearic esters.' *Arch. Biochem. Biophys.*, 1992, **294**, 675–685.
22. P. P. Halarnkar, R. N. Wixtrom, M. H. Silva *et al.*, 'Catabolism of epoxy fatty esters by the purified epoxide hydrolase from mouse and human liver.' *Arch. Biochem. Biophys.*, 1989, **272**, 226–236.

23. P. P. Halarnkar, J. Nourooz-Zadeh, E. Kuwano *et al.*, 'Formation of cyclic products from the diepoxide of long-chain fatty esters by cytosolic epoxide hydrolase.' *Arch. Biochem. Biophys.*, 1992, **294**, 586–593.
24. B. Borhan, J. Nourooz-Zadeh, T. Uematsu *et al.*, 'Stereochemical aspects of cytosolic epoxide hydrolase hydration of methyl diepoxystearates.' *Tetrahedron*, 1993, **49**, 2601.
25. D. C. Zeldin, J. Kobayashi, J. R. Falck *et al.*, 'Regio- and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase.' *J. Biol. Chem.*, 1993, **268**, 6402–6407.
26. D. C. Zeldin, S. Wei, J. R. Falck *et al.*, 'Metabolism of epoxyeicosatrienoic acids by cytosolic epoxide hydrolase: substrate structural determinants of asymmetric catalysis.' *Arch. Biochem. Biophys.*, 1995, **316**, 443–451.
27. M. F. Moghaddam, K. Motoba, B. Borhan *et al.*, 'Novel microsomal pathway for linoleic and arachidonic acid metabolism.' *Biochem. Biophys. Acta*, 1996, **1290**, 327–339.
28. E. Blee and F. Schuber, 'Regio- and enantioselectivity of soybean fatty acid epoxide hydrolase.' *J. Biol. Chem.*, 1992, **267**, 11881–11887.
29. A. Wetterholm, J. Haeggstrom, M. Hamberg *et al.*, '14,15-Dihydroxy-5,8,10,12-eicosatetraenoic acid. Enzymatic formation from 14,15-leukotriene A_4.' *Eur. J. Biochem.*, 1988, **173**, 531–536.
30. J. Haeggstrom, A. Wetterholm, M. Hamberg *et al.*, 'Enzymatic formation of 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid: kinetics of the reaction and stereochemistry of the product.' *Biochim. Biophys. Acta*, 1988, **958**, 469–476.
31. I. Miki, T. Shimizu, Y. Seyama *et al.*, 'Enzymic conversion of 11,12-leukotriene A_4 to 11,12-dihydroxy-5,14-*cis*-7,9-*trans*-eicosatetraenoic acid. Purification of an epoxide hydrolase from the guinea pig liver cytosol.' *J. Biol. Chem.*, 1989, **264**, 5799–5805.
32. K. Kosaka, K. Suzuki, M. Hayakawa *et al.*, 'Leukotoxin, a linoleate epoxide: its implication in the late death of patients with extensive burns.' *Mol. Cell. Biochem.*, 1994, **139**, 141–148.
33. M. Moghaddam, D. Grant, J. Cheek *et al.*, 'Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase.' *Nature Medicine*, 1996, in press.
34. B. Borhan, T. Mebrahtu, S. Nazarian *et al.*, 'Improved radiolabeled substrates for soluble epoxide hydrolase.' *Anal. Biochem.*, 1995, **231**, 188–200.
35. B. D. Hammock, D. E. Moody and A. Sevanian, in 'Methods in Enzymology, Volume III, Steriods and Isoprenoids, Part B,' eds. J. H. Law and H. C. Rilling, Academic Press, FL, 1985, pp. 303–311.
36. S. S. Gill, K. Ota, B. Ruebner *et al.*, 'Microsomal and cytosolic epoxide hydrolases in rhesus monkey liver, and in normal and neoplastic human liver.' *Life Sci.*, 1983, **32**, 2693–2706.
37. D. L. Eaton and P. L. Stapleton, 'Simultaneous determination of cytosolic GSH Stransferase and microsomal epoxide hydrolase activity toward benzo-(*a*)pyrene-4,5-oxide by high-performance liquid chromatography.' *Anal. Biochem.*, 1989, **178**, 153–158.
38. F. Oesch, D. M. Jerina and J. Daly, 'A radiometric assay for hepatic epoxide hydrase activity with 7-[^{3}H] styrene oxide.' *Biochim. Biophys. Acta*, 1971, **227**, 685–691.
39. G. T. Brooks, 'Insect epoxide hydrase inhibition by juvenile hormone analogues and metabolic inhibitors.' *Nature*, 1973, **245**, 382.
40. F. Oesch, D. M. Jerina and J. W. Daly, 'Substrate specificity of hepatic epoxide hydrase in microsomes and in a purified preparation: evidence for homologous enzymes.' *Arch. Biochem. Biophys.*, 1971, **144**, 253–261.
41. S. M. Mumby and B. D. Hammock, 'Substrate selectivity and stereochemistry of enzymatic epoxide hydration in the soluble fraction of mouse liver.' *Pestic. Biochem. Physiol.*, 1979, **11**, 275–284.
42. B. D. Hammock and L. S. Hasagawa, 'Differential substrate selectivity of murine hepatic cytosolic and microsomal epoxide hydrolases.' *Biochem. Pharmacol.*, 1983, **32**, 1155–1164.
43. T. A. Stoming and E. Bresnick, 'Gas chromatographic assay of epoxide hydrase activity with 3-methylcholanthrene-11,12-oxide.' *Science*, 1973, **181**, 951–952.
44. D. M. Jerina and P. M. Dansette 'Hepatic microsomal epoxide hydrase: a sensitive radiometric assay for hydration of arene oxides of carcinogenic aromatic hydrocarbons.' *Mol. Pharmacol.*, 1977, **13**, 342–351.
45. F. Oesch, N. Kaubisch, D. M. Jerina *et al.*, 'Hepatic epoxide hydrase. Structure-activity relationships for substrates and inhibitors.' *Biochemistry*, 1971, **10**, 4858–4866.
46. F. Oesch, D. Beermann, A. J. Sparrow *et al.*, 'A highly sensitive assay for epoxide hydrolase using an endogenous epoxide as substrate: 16α,17α-epoxyandrost-4-en-3-one.' *Anal. Biochem.*, 1981, **117**, 223–230.
47. K. A. Giuliano, E. P. Lau and R. R. Fall, 'Simplified liquid chromatographic assay for epoxide hydrolase.' *J. Chromatogr.*, 1980, **202**, 447–452.
48. R. B. Westkaemper and R. P. Hanzlik, 'A convenient reverse-phase liquid chromatographic assay for epoxide hydrase.' *Anal. Biochem.*, 1980, **102**, 63–67.
49. S. Nesnow and C. Heidelberger, 'A rapid and sensitive liquid chromatographic assay for epoxide hydrase.' *Anal. Biochem.*, 1975, **67**, 525–530.
50. S. S. Gill, K. Ota and B. D. Hammock, 'Radiometric assays for mammalian epoxide hydrolases and GSH Stransferase.' *Anal. Biochem.*, 1983, **131**, 273–282.
51. B. D. Hammock, D. N. Loury, D. E. Moody *et al.*, 'A methodology for the analysis of the preneoplastic antigen.' *Carcinogenesis*, 1984, **5**, 1467–1473.
52. W. Watabe and K. Akamatsu, 'Photometric assay of hepatic epoxide hydrolase activity with safrole oxide (SAFO) as substrate.' *Biochem. Pharmacol.*, 1974, **23**, 2839–2844.
53. H. U. Schmassmann, H. R. Glatt and F. Oesch, 'A rapid assay for epoxide hydratase activity with benzo(*a*)pyrene 4,5-(K-region)-oxide as substrate.' *Anal. Biochem.*, 1976, **74**, 94–104.
54. P. Bentley, H. Schmassmann, P. Sims *et al.*, 'Epoxides derived from various polycyclic hydrocarbons as substrates of homogeneous and microsome-bound epoxide hydrolase. A general assay and kinetic properties.' *Eur. J. Biochem.*, 1976, **69**, 97–103.
55. U. Bindel, A. Sparrow, H. Schmassmann *et al.*, 'Endogenous role of epoxide hydratase. Development of a steroid epoxide-hydratase assay and properties of the enzyme.' *Eur. J. Biochem.*, 1979, **97**, 275–281.
56. R. N. Armstrong, W. Levin and D. M. Jerina, in 'Microsomes, Drug Oxidations, Chemical Carcinogenesis,' eds. M. Coon, A. Conney, R. Estabrook *et al.*, Academic Press, New York, 1980, vol. II, pp. 1119–1123.
57. P. M. Dansette, G. C. DuBois and D. M. Jerina, 'Continuous fluorometric assay of epoxide hydrase activity.' *Anal. Biochem.*, 1979, **97**, 340–345.
58. M. W. Hukkelhoven, E. W. Vromans, A. J. Vermorken *et al.*, 'A sensitive fluorometric assay for epoxide hydratase.' *FEBS Lett.*, 1982, **144**, 104–108.
59. F. P. Guengerich and P. S. Mason, 'Alcohol dehydrogenase-coupled spectrophotometric assay of epoxide hydratase activity.' *Anal. Biochem.*, 1980, **104**, 445–451.

60. R. B. Westkaemper and R. P. Hanzlik, 'Mechanistic studies of epoxide hydrolase utilizing continuous spectrophotometric assay.' *Arch. Biochem. Biophys.*, 1981, **208**, 195–204.
61. R. N. Wixtrom and B. D. Hammock, 'Continuous spectrophotometric assays for cytosolic epoxide hydrolase.' *Anal. Biochem.*, 1988, **174**, 291–299.
62. T. Sone, M. Isobe, E. Takabatake *et al.*, '7-Ethenyloxycoumarin as a new substrate for fluorophotometric assay of hepatic microsomal epoxidizing activities.' *J. Pharmacobiodyn.*, 1989, **12**, 149–158.
63. S. M. Mumby and B. D. Hammock, 'A partition assay for epoxide hydrases acting on insect juvenile hormone and an epoxide-containing juvenoid.' *Anal. Biochem.*, 1979, **92**, 16–21.
64. C. A. Mullin and B. D. Hammock, 'A rapid radiometric assay for mammalian cytosolic epoxide hydrolase.' *Anal. Biochem.*, 1980, **106**, 476–485.
65. L. S. Hasegawa and B. D. Hammock, 'Spectrophotometric assay for mammalian cytosolic epoxide hydrolase using *trans*-stilbene oxide as the substrate.' *Biochem. Pharmacol.*, 1982, **31**, 1979–1984.
66. E. C. Dietze, E. Kuwano and B. D. Hammock, 'Spectrophotometric substrates for cytosolic epoxide hydrolase.' *Anal. Biochem.*, 1994, **216**, 176–187.
67. J. G. Pritchard and F. A. Long, 'Kinetics and mechanism of the acid-catalyzed hydrolysis of substituted ethylene.' *J. Am. Chem. Soc.*, 1956, **78**, 2667.
68. G. M. Lacourciere and R. N. Armstrong, 'The catalytic mechanism of microsomal epoxide hydrolase involves an ester intermediate.' *J. Am. Chem. Soc.*, 1993, **115**, 10466.
69. M. Arand, D. F. Grant, J. K. Beetham *et al.*, 'Sequence similarity of mammalian epoxide hydrolases to the bacterial haloalkane dehalogenase and other related proteins. Implication for the potential catalytic mechanism of enzymatic epoxide hydrolase.' *FEBS Lett.*, 1994, **338**, 251–256.
70. R. P. Hanzlik, M. Edelman, W. J. Michaely *et al.*, 'Enzymatic hydration of ^{18}O-epoxides. Role of nucleophilic mechanisms.' *J. Am. Chem. Soc.*, 1976, **98**, 1952–1955.
71. R. N. Armstrong, W. Levin and D. M. Jerina, 'Hepatic microsomal epoxide hydrolase. Mechanistic studies of the hydration of K-region arene oxides.' *J. Biol. Chem.*, 1980, **255**, 4698–4705.
72. G. T. Brooks, S. E. Lewis and A. Harrison, 'Selective metabolism of cyclodiene insecticide enantiomers by pig liver microsomal enzymes.' *Nature*, 1968, **220**, 1034–1035.
73. P. M. Dansette, V. B. Makedonska and D. M. Jerina, 'Mechanism of catalysis for the hydration of substituted styrene oxides by hepatic epoxide hydrase.' *Arch. Biochem. Biophys.*, 1978, **187**, 290–298.
74. R. P. Hanzlik and R. B. Westkaemper, 'Secondary deuterium isotope effects on epoxide methanolysis reactions.' *J. Am. Chem. Soc.*, 1980, **102**, 2464–2467.
75. G. Bellucci, I. Capitani, C. Chiappe *et al.*, 'Product enantioselectivity of the microsomal and cytosolic epoxide hydrolase catalysed hydrolysis of *meso* epoxides.' *J. Chem. Soc., Chem. Commun.*, 1989, **16**, 1170.
76. G. Bellucci, C. Chiappe, F. Marioni *et al.*, 'The course of the microsomal epoxide hydrolase-catalyzed and acid-catalyzed hydrolysis of (−−)-2-methyl-5*H*-dibenzo[*a,d*]cycloheptene 10,11-oxide.' *Societa Chimica Italiana*, 1992, **122**, 1.
77. B. D. Hammock, M. Ratcliff and D. A. Schooley, 'Hydration of an ^{18}O epoxide by a cytosolic epoxide hydrolase from mouse liver.' *Life Sci.*, 1980, **27**, 1635–1641.
78. G. C. DuBois, E. Appella, W. Levin *et al.*, 'Hepatic microsomal epoxide hydrase. Involvement of a histidine at the active site suggests a nucleophilic mechanism.' *J. Biol. Chem.*, 1978, **253**, 2932–2939.
79. S. Elsberg, H. Thomas, H. D. Zeller *et al.*, in 'VIIIth International Symposium on Microsomes and Drug Oxidations, Karolinska Instituet, 1990,' eds. M. Ingelman-Sundberg, J.-A. Gustafsson and S. Orrenius, Kongl Carolinska Medico Chirurgiska Institutet, Stockholm, 1990, p. 126.
80. P. A. Bell and C. B. Kasper, 'Expression of rat microsomal epoxide hydrolase in *Escherichia coli*. Identification of a histidyl residue essential for catalysis.' *J. Biol. Chem.*, 1993, **268**, 14011–14017.
81. D. B. Janssen, F. Pries, J. Vander Ploeg *et al.*, 'Cloning of 1,2-dichloroethane degradation genes of *Xanthobacter autotrophicus* GJ10 and expression and sequencing of the *dhlA* gene.' *J. Bacteriol.*, 1989, **171**, 6791–6799.
82. K. H. Verschueren, F. Seljee, H. J. Rozeboom *et al.*, 'Crystallographic analysis of the catalytic mechanism of haloalkane dehalogenase.' *Nature*, 1993, **363**, 693–698.
83. B. Borhan, A. D. Jones, F. Pinot *et al.*, 'Mechanism of soluble epoxide hydrolase. Formation of an α-hydroxy ester-enzyme intermediate through Asp-333.' *J. Biol. Chem.*, 1995, **270**, 26923–26930.
84. B. D. Hammock, F. Pinot, J. K. Beetham *et al.*, 'Isolation of a putative hydroxyacyl enzyme intermediate of an epoxide hydrolase.' *Biochem. Biophys. Res. Commun.*, 1994, **198**, 850–856.
85. F. Pinot, D. F. Grant, J. K. Beetham *et al.*, 'Molecular and biochemical evidence for the involvement of the Asp-333–His-523 pair in the catalytic mechanism of soluble epoxide hydrolase.' *J. Biol. Chem.*, 1995, **270**, 7968–7974.
86. B. Borhan, Y. Ko, C. Mackay *et al.*, 'Development of surrogate substrates for neuropathy target esterase.' *Biochim. Biophys. Acta*, 1995, **1250**, 171–182.
87. M. Arand, H. Wagner and F. Oesch, 'Asp333, Asp495, and His523 form the catalytic triad of rat soluble epoxide hydrolase.' *J. Biol. Chem.*, 1996, **271**, 4223–4229.
88. J. K. Beetham, D. Grant, M. Arand *et al.*, 'Gene evolution of epoxide hydrolases and recommended nomenclature.' *DNA Cell Biol.*, 1995, **14**, 61–71.
89. D. L. Ollis, E. Cheah, M. Cygler *et al.*, 'The α/β hydrolase fold.' *Protein Eng.*, 1992, **5**, 197.
90. D. M. Blow, J. J. Birktoft and B. S. Hartley, 'Role of a buried acid group in the mechanism of action of chymotrypsin.' *Nature*, 1969, **221**, 337–340.
91. W. Levin, P. E. Thomas, D. Korzeniowski *et al.*, 'Liver microsomal epoxide hydrase: activation, inhibition, and immunological properties of the purified enzyme.' *Mol. Pharmacol.*, 1978, **14**, 1107–1120.
92. D. Guest and J. G. Dent, 'Effects of epoxide hydratase inhibitors in forward and reversion bacterial mutagenesis assay systems.' *Environ. Mutagen.*, 1980, **2**, 27–34.
93. J. Magdalou and B. D. Hammock, '1,2-Epoxycycloalkanes: substrates and inhibitors of microsomal and cytosolic epoxide hydrolases in mouse liver.' *Biochem. Pharmacol.*, 1988, **37**, 2717–2722.
94. R. P. Hanzlik and J. S. Walsh, 'Halogenated epoxides and related compounds as inhibitors of epoxide hydrolase.' *Arch. Biochem. Biophys.*, 1980, **204**, 255–263.
95. A. C. C. Craven, G. T. Brooks and C. H. Walker, 'The inhibition of HEOM epoxide hydrase in mammalian liver microsomes and insect pupal homogenates.' *Pestic. Biochem. Physiol.*, 1976, **6**, 132–141.
96. C. A. Mullin and B. D. Hammock, 'Chalcone oxides — potent selective inhibitors of cytosolic epoxide hydrolase.' *Arch. Biochem. Biophys.*, 1982, **216**, 423–439.

97. R. Wolfenden, 'Transition state analog inhibitors and enzyme catalysis.' *Annu. Rev. Biophys. Bioeng.*, 1976, **45**, 271-306.
98. B. D. Hammock, G. D. Prestwich, D. N. Loury *et al.*, 'Comparison of crude and affinity purified cytosolic epoxide hydrolases from hepatic tissue of control and clofibrate-fed mice.' *Arch. Biochem. Biophys.*, 1986, **244**, 292-309.
99. E. C. Dietze, E. Kuwano, J. Casas *et al.*, 'Inhibition of cytosolic epoxide hydrolase by *trans*-3-phenylglycidols.' *Biochem. Pharmacol.*, 1991, **42**, 1163-1175.
100. T. Miyamoto, M. Silva and B. D. Hammock, 'Inhibition of epoxide hydrolases and GSH *S*transferases by 2-, 3-, and 4-substituted derivatives of 4'-phenylchalcone and its oxide.' *Arch. Biochem. Biophys.*, 1987, **254**, 203-213.
101. E. C. Dietze, J. Casas, E. Kuwano *et al.*, 'Inhibition of epoxide hydrolase from human, monkey, bovine, rabbit, and murine liver by *trans*-3-phenylglycidols.' *Comp. Biochem. Physiol. B*, 1993, **104**, 309-314.
102. E. C. Dietze, E. Kuwano and B. D. Hammock, 'The interaction of cytosolic epoxide hydrolase with chiral epoxides.' *Int. J. Biochem.*, 1993, **25**, 43-52.
103. D. A. Svinarov and C. E. Pippenger, 'Valproic acid-carbamazepine interaction: is valproic acid a selective inhibitor or epoxide hydrolase?' *Therap. Drug Monit.*, 1995, **17**, 217-220.
104. I. Mertes, R. Fleischmann, H. R. Glatt *et al.*, 'Interindividual variations in the activities of cytosolic and microsomal epoxide hydrolase in human liver.' *Carcinogenesis*, 1985, **6**, 219-223.
105. F. Oesch, H. Glatt and H. Schimassmann, 'The apparent ubiquity of epoxide hydratase in rat organs.' *Biochem. Pharmacol.*, 1977, **26**, 603-607.
106. D. Papadopoulos, H. Jornvall, J. Rydstrom *et al.*, 'Purification and initial characterization of microsomal epoxide hydrolase from the human adrenal gland.' *Biochim. Biophys. Acta*, 1994, **1206**, 253-262.
107. E. C. Dietze, J. Stephens, J. Magdalou *et al.*, 'Inhibition of human and murine cytosolic epoxide hydrolase by group-selective reagents.' *Comp. Biochem. Physiol. B*, 1993, **104**, 299-308.
108. G. Bellucci, C. Chiappe, A. Cordoni *et al.*, 'The rabbit liver microsomal biotransformation of 1,1-dialkylethylenes: enantioface selection of epoxidation and enantioselectivity of epoxide hydrolysis.' *Chirality*, 1994, **6**, 207-212.
109. C. Hassett, S. M. Turnblom, A. DeAngles *et al.*, 'Rabbit microsomal epoxide hydrolase: isolation and characterization of the xenobiotic metabolizing enzyme cDNA.' *Arch. Biochem. Biophys.*, 1989, **271**, 380-389.
110. N. Ariyoshi, M. Tanaka, Y. Ishii *et al.*, 'Purification and characterization of dog liver microsomal epoxide hydrolase.' *J. Biochem. (Tokyo)*, 1994, **115**, 985-990.
111. D. M. Gillette, R. D. Corey, W. G. Helferich *et al.*, 'Comparative toxicology of tetrachlorobiphenyls in mink and rat. I. Changes in hepatic enzyme activity and smooth endoplasmic reticulum volume.' *Fundam. Appl. Toxicol.*, 1987, **8**, 5-14.
112. J. A. Wisniewski, D. E. Moody, B. D. Hammock *et al.*, 'Interlobular distribution of hepatic xenobiotic-metabolizing enzyme activities in cattle, goats and sheep.' *J. Anim. Sci.*, 1987, **64**, 210-215.
113. D. J. Lauren, P. P. Halarnkar, B. D. Hammock *et al.*, 'Microsomal and cytosolic epoxide hydrolase, and GSH *S*-transferase activities in the gill, liver, and kidney of the rainbow trout, *Salmo gairdneri*. Baseline levels and optimization of assay conditions.' *Biochem. Pharmacol.*, 1989, **38**, 881-887.
114. F. Waechter, P. Bentley, F. Bieri *et al.*, 'Organ distribution of epoxide hydrolases in cytosolic and microsomal fractions of normal and nafenopin-treated male DBA 2 mice.' *Biochem. Pharmacol.*, 1988, **37**, 3897-3903.
115. S. Petruzzelli, M. Franchi, L. Gronchi *et al.*, 'Cigarette smoke inhibits cytosolic but not microsomal epoxide hydrolase of human lung.' *Hum. Exp. Toxicol.*, 1992, **11**, 99-103.
116. J. Seidegard, J. W. DePierre and R. W. Pero, 'Measurement and characterization of membrane-bound and soluble epoxide hydrolase activities in resting mononuclear leukocytes from human blood.' *Cancer Res.*, 1984, **44**, 3654-3660.
117. W. Honscha, F. Oesch and T. Friedberg, 'Tissue-specific expression and differential inducibility of several microsomal epoxide hydrolase mRNAs which are formed by alternative splicing.' *Arch. Biochem. Biophys.*, 1991, **287**, 380-385.
118. V. D. Ding, R. Cameron and C. B. Pickett, 'Regulation of microsomal, xenobiotic epoxide hydrolase messenger RNA in persistent hepatocyte nodules and hepatomas induced by chemical carcinogens.' *Cancer Res.*, 1990, **50**, 256-260.
119. S. Kondo, B. I. Carr, K. Takagi *et al.*, 'Expression of rat microsomal epoxide hydrolase gene during liver chemical carcinogenesis.' *Cancer. Res.*, 1990, **50**, 6222-6228.
120. L. Massaad, I. de Waziers, V. Ribrag *et al.*, 'Comparison of mouse and human colon tumors with regard to phase I and phase II drug-metabolizing enzyme systems.' *Cancer Res.*, 1992, **52**, 6567-6575.
121. C. Toussaint, N. Albin, L. Massaad *et al.*, 'Main drug- and carcinogen-metabolizing enzyme systems in human non-small cell lung cancer and peritumoral tissues.' *Cancer Res.*, 1993, **53**, 4608-4612.
122. F. Janot, L. Massaad, V. Ribrag *et al.*, 'Principal xenobiotic-metabolizing enzyme systems in human head and neck squamous cell carcinoma.' *Carcinogenesis*, 1993, **14**, 1279-1283.
123. N. Albin, L. Massaad, C. Toussaint *et al.*, 'Main drug-metabolizing enzyme systems in human breast tumors and peritumoral tissues.' *Cancer Res.*, 1993, **53**, 3541-3546.
124. G. I. Murray, R. J. Weaver, P. J. Paterson *et al.*, 'Expression of xenobiotic metabolizing enzymes in breast cancer.' *J. Pathol.*, 1993, **169**, 347-353.
125. G. I. Murray, P. J. Paterson, R. J. Weaver *et al.*, 'The expression of cytochrome P-450, epoxide hydrolase, and GSH transferase in hepatocellular carcinoma.' *Cancer*, 1993, **71**, 36-43.
126. D. Papadopoulos, S. Grondal, J. Rydstrom *et al.*, 'Levels of cytochrome P-450, steroidogenesis and microsomal and cytosolic epoxide hydrolases in normal human adrenal tissue and corresponding tumors.' *Cancer Biochem. Biophys.*, 1992, **12**, 283-291.
127. J. A. McKay, R. J. Weaver, G. I. Murray *et al.*, 'Localization of microsomal epoxide hydrolase in normal and neoplastic human kidney.' *J. Histochem. Cytochem.*, 1995, **43**, 615-620.
128. H. Glatt, I. Gemperlein, F. Setiabudi *et al.*, 'Expression of xenobiotic-metabolizing enzymes in propagatable cell cultures and induction of micronuclei by 13 compounds.' *Mutagenesis*, 1990, **5**, 241-249.
129. P. Steinberg, R. Steinbrecher, D. Schrenk *et al.*, 'Drug-metabolizing enzyme activities in freshly isolated oval cells and in an established oval cell line from carcinogen-fed rats.' *Cell Biol. Toxicol.*, 1994, **10**, 59-65.
130. A. M. Pfeifer, K. E. Cole, D. T. Smoot *et al.*, 'Simian virus 40 large tumor antigen-immortalized normal human liver epithelial cells express hepatocyte characteristics and metabolize chemical carcinogens.' *Proc. Natl. Acad. Sci. U.S.A.*, 1993, **90**, 5123-5127.

131. F. Oesch, L. Schladt, R. Hartmann *et al.*, 'Rat cytosolic epoxide hydrolase.' *Adv. Exp. Med. Biol.*, 1986, **197**, 195–201.

132. L. Schladt, W. Worner, F. Setiabudi *et al.*, 'Distribution and inducibility of cytosolic epoxide hydrolase in male Sprague-Dawley rats.' *Biochem. Pharmacol.*, 1986, **35**, 3309–3316.

133. S. S. Gill and B. D. Hammock, 'Distribution and properties of a mammalian soluble epoxide hydrase.' *Biochem. Pharmacol.*, 1980, **29**, 389–395.

134. K. W. DiBiasio, M. H. Silva, B. D. Hammock *et al.*, 'Effects of hepatic inducers on testicular epoxide-metabolizing enzymes in the rat and mouse.' *Fundam. Appl. Toxicol.*, 1989, **12**, 449–459.

135. M. H. Silva, R. N. Wixtrom and B. D. Hammock, 'Epoxide-metabolizing enzymes in mammary gland and liver from BALB c mice and effects of inducers on enzyme activity.' *Cancer Res.*, 1988, **48**, 1390–1397.

136. G. M. Pacifici, A. Temellini, L. Giuliani *et al.*, 'Cytosolic epoxide hydrolase in humans: development and tissue distribution.' *Arch. Toxicol.*, 1988, **62**, 254–257.

137. M. A. Pham, J. Magdalou, M. Totis *et al.*, 'Characterization of distinct forms of cytochromes P-450, epoxide metabolizing enzymes and UDP-glucuronosyltransferases in rat skin.' *Biochem. Pharmacol.*, 1989, **38**, 2187–2194.

138. B. S. Winder, J. Nourooz-Zadeh, R. R. Isseroff *et al.*, 'Properties of enzymes hydrating epoxides in human epidermis and liver.' *Int. J. Biochem.*, 1993, **25**, 1291–1301.

139. D. Utesch, M. Arand, H. Thomas, E. Petzinger *et al.*, 'Xenobiotic-metabolizing enzyme activities in hybrid cell lines established by fusion of primary rat liver parenchymal cells with hepatoma cells.' *Xenobiotica*, 1992, **22**, 1451–1457.

140. M. Roques, D. Bagrel, J. Magdalou *et al.*, 'Expression of arylhydrocarbon hydroxylase, epoxide hydrolases, glutatione *S*-transferase and UDP-glucuronosyltransferases in H5-6 hepatoma cells.' *Gen. Pharmacol.*, 1991, **22**, 677–684.

141. A. Kramer, H. Frank, F. Setiabudi *et al.*, 'Influence of the level of cytosolic epoxide hydrolase on the induction of sister chromatid exchanges by *trans*-β-ethylstyrene 7,8-oxide in human lymphocytes.' *Biochem. Pharmacol.*, 1991, **42**, 2147–2152.

142. A. Kramer, J. Pudil, H. Frank *et al.*, 'Some substrates and inhibitors of cytosolic epoxide hydrolase induce sister-chromatid exchanges in mammalian cells, but do not induce gene mutations in *Salmonella typhimurium* and V79 cells.' *Mutat. Res.*, 1993, **290**, 165–174.

143. B. D. Hammock and K. Ota, 'Differential induction of cytosolic epoxide hydrolase, microsomal epoxide hydrolase, and GSH transferase activities.' *Toxicol. Appl. Pharmacol.*, 1983, **71**, 254–265.

144. D. E. Moody and B. D. Hammock, 'Purification of microsomal epoxide hydrolase from liver of rhesus monkey: partial separation of *cis*- and *trans*-stilbene oxide hydrolase.' *Arch. Biochem. Biophys.*, 1987, **258**, 156–164.

145. F. P. Guengerich, P. Wang, M. B. Mitchell *et al.*, 'Rat and human liver microsomal epoxide hydratase. Purification and evidence for the existence of multiple forms.' *J. Biol. Chem.*, 1979, **254**, 12248–12254.

146. F. P. Guengerich, P. Wang, P. S. Mason *et al.*, 'Rat and human microsomal epoxide hydratase. Immunological characterization of various forms of the enzyme.' *J. Biol. Chem.*, 1979, **254**, 12255–12259.

147. N. J. Bulleid, A. B. Graham and J. A. Craft, 'Microsomal epoxide hydrolase of rat liver. Purification and characterization of enzyme fractions with different chromatographic characteristics.' *Biochem. J.*, 1986, **233**, 607–611.

148. P. H. Beaune, T. Crestell, J. P. Flinois *et al.*, 'Hydrophobic chromatography: a one-step method for the purification of human liver microsomal epoxide hydrolase.' *J. Chromatogr.*, 1988, **426**, 169–176.

149. T. D. Porter, T. W. Beck and C. B. Kasper, 'Complementary DNA and amino acid sequence of rat liver microsomal, xenobiotic epoxide hydrolase.' *Arch. Biochem. Biophys.*, 1986, **248**, 121–129.

150. J. A. Craft, S. Baird, M. Lamont *et al.*, 'Membrane topology of epoxide hydrolase.' *Biochim. Biophys. Acta*, 1990, **1046**, 32–39.

151. T. Friedberg, B. Lollmann, R. Becker *et al.*, 'The microsomal epoxide hydrolase has a single membrane signal anchor sequence which is dispensable for the catalytic activity of this protein.' *Biochem. J.*, 1994, **303**, 967–972.

152. C. Alves, P. von Dippe, M. Amoui *et al.*, 'Bile acid transport into hepatocyte smooth endoplasmic reticulum vesicles is mediated by microsomal epoxide hydrolase, a membrane protein exhibiting two distinct topological orientations.' *J. Biol. Chem.*, 1993, **268**, 20148–20155.

153. M. J. Griffin and N. Gengozian, 'Epoxide hydrolase: a marker for experimental hepatocarcinogenesis.' *Ann. Clin. Lab. Sci.*, 1984, **14**, 27–31.

154. D. E. Moody, D. N. Loury, B. D. Hammock *et al.*, 'Serum epoxide hydrolase (preneoplastic antigen) in human and experimental liver injury.' *Cancer Epidemiol. Biomarkers Prev.*, 1992, **1**, 395–403.

155. R. C. Skoda, A. Demierre, O. W. McBride *et al.*, 'Human microsomal xenobiotic epoxide hydrolase. Complementary DNA sequence, complementary DNA-directed expression in COS-1 cells, and chromosomal localization.' *J. Biol. Chem.*, 1988, **263**, 1549–1554.

156. M. R. Jackson and B. Burchell, 'Expression of human liver epoxide hydrolase in *Saccharomyces pombe*.' *Biochem. J.*, 1988, **251**, 931–933.

157. G. M. Lacourciere, V. N. Vakharia, C. P. Tan *et al.*, 'Interaction of hepatic microsomal epoxide hydrolase derived from a recombinant baculovirus expression system with a azarene oxide and an aziridine substrate analogue.' *Biochemistry*, 1993, **32**, 2610–2616.

158. H. P. Eugster, C. Sengstag, A. Hinnen *et al.*, 'Heterologous expression of human microsomal epoxide hydrolase in *Saccharomyces cerevisiae*. Study of the valpromide–carbamazepine epoxide interaction.' *Biochem. Pharmacol.*, 1991, **42**, 1367–1372.

159. J. C. Gautier, P. Urban, P. Beaune *et al.*, 'Engineered yeast cells as model to study coupling between human xenobiotic metabolizing enzymes. Simulation of the two first steps of benzo(*a*)pyrene activation.' *Eur. J. Biochem.*, 1993, **211**, 63–72.

160. C. Crofton-Sleigh, A. Doherty, S. Ellard *et al.*, 'Micronucleus assays using cytochalasin-blocked MCL-5 cells, a proprietary human cell line expressing five human cytochromes P-450 and microsomal epoxide hydrolase.' *Mutagenesis*, 1993, **8**, 363–372.

161. W. F. Busby, Jr., H. Smith, C. L. Crespi *et al.*, 'Mutagenicity of benzo(*a*)pyrene and dibenzopyrenes in the *Salmonella typhimurium* TM677 and the MCL-5 human cell forward mutation assays.' *Mutat. Res.*, 1995, **342**, 9–16.

162. R. L. Davies, C. L. Crespi, K. Rudo *et al.*, 'Development of a human cell line by selection and drug-metabolizing gene transfection with increased capacity to activate promutagens.' *Carcinogenesis*, 1989, **10**, 885–891.

163. C. L. Crespi, F. J. Gonzalez, D. T. Steimel *et al.*, 'A metabolically competent human cell line expressing five cDNAs encoding procarcinogen-activating enzymes: application to mutagenicity testing.' *Chem. Res. Toxicol.*, 1991, **4**, 566–572.

164. C. L. Crespi, R. Langenbach and B. W. Penman, 'Human cell lines, derived from AHH-1 TK⁻ human lymphoblasts, genetically engineered for expression of cytochromes P450.' *Toxicology*, 1993, **82**, 89–104.

165. W. F. Busby, Jr., B. W. Penman and C. L. Crespi, 'Human cell mutagenicity of mono- and dinitropyrenes in metabolically competent MCL-5 cells.' *Mutat. Res.*, 1994, **322**, 233–242.

166. M. Shou, K. R. Korzekwa, C. L. Crespi *et al.*, 'The role of 12 cDNA-expressed human, rodent, and rabbit cytochromes P450 in the metabolism of benzo(*a*)pyrene and benzo(*a*)pyrene trans-7,8-dihydrodiol.' *Mol. Carcinog.*, 1994, **10**, 159–168.

167. T. Friedberg, R. Becker, F. Oesch *et al.*, 'Studies on the importance of microsomal epoxide hydrolase in the detoxification of arene oxides using the heterologous expression of the enzyme in mammalian cells.' *Carcinogenesis*, 1994, **15**, 171–175.

168. J. M. Sayer, H. Yagi, P. J. van Bladeren *et al.*, 'Stereoselectivity of microsomal epoxide hydrolase toward diol epoxides and tetrahydroepoxides derived from benz(*a*)anthracene.' *J. Biol. Chem.*, 1985, **260**, 1630–1640.

169. C. N. Falany, P. McQuiddy and C. B. Kasper, 'Structure and organization of the microsomal xenobiotic epoxide hydrolase gene.' *J. Biol. Chem.*, 1987, **262**, 5924–5930.

170. J. H. Nadeau, M. T. Davisson, D. P. Doolittle *et al.*, 'Comparative map for mice and humans.' *Mamm. Genome.*, 1992, **3**, 480–534.

171. C. Hassett, K. Robinson, N. Beck *et al.*, 'The human microsomal epoxide hydrolase gene (EPHX1): complete nucleotide sequence and structural characterization.' *Genomics*, 1994, **23**, 433–442.

172. S. Kondo, P. N. Gertson, K. Yokoyama *et al.*, 'Hepatic transcriptional up-regulator of the rat microsomal epoxide hydrolase gene.' *DNA Cell Biol.*, 1994, **13**, 43–50.

173. S. Kondo, Y. Chou, P. N. Gertson *et al.*, 'A bipartite suppressor: conjunction of two distinct factor-binding sites is essential for down-regulation in rat epoxide hydrolase gene expression.' *Mol. Cell. Biol.*, 1992, **12**, 4496–4502.

174. P. A. Bell, C. N. Falany, P. McQuiddy *et al.*, 'Glucocorticoid repression and basal regulation of the epoxide hydrolase promoter.' *Arch. Biochem. Biophys.*, 1990, **279**, 363–369.

175. B. A. Buehler, D. Delimont, M. van Waes *et al.*, 'Prenatal prediction of risk of the fetal hydantoin syndrome.' *N. Engl. J. Med.*, 1990, **322**, 1567–1572.

176. A. Gaedigk, S. P. Spielberg and D. M. Grant, 'Characterization of the microsomal epoxide hydrolase gene in patients with anticonvulsant adverse drug reactions.' *Pharmacogenetics*, 1994, **4**, 142–153.

177. C. Hassett, L. Aicher, J. S. Sidhu *et al.*, 'Human microsomal epoxide hydrolase: genetic polymorphism and functional expression *in vitro* of amino acid variants.' *Hum. Mol. Genet.*, 1994, **3**, 421–428.

178. A. D. Vaz, V. M. Fiorica and M. J. Griffin, 'New heterocyclic stimulators of hepatic epoxide hydrolase.' *Biochem. Pharmacol.*, 1981, **30**, 651–656.

179. J. Seidegard and J. W. DePierre, 'Benzil, a potent activator of microsomal epoxide hydrolase *in vitro*.' *Eur. J. Biochem.*, 1980, **112**, 643–648.

180. V. S. Ganu and W. L. Alworth, 'Epoxide hydrase: structure-activity relationships of *in vitro* stimulators of the microsomal activity.' *Biochemistry*, 1978, **17**, 2876–2881.

181. W. L. Alworth, C. C. Dang, L. M. Ching *et al.*, 'Stimulation of mammalian epoxide hydrase activity by flavones.' *Xenobiotica*, 1980, **10**, 395–400.

182. J. Seidegard, J. W. DePierre, T. M. Guenthner *et al.*, 'The effects of metyrapone, chalcone epoxide, benzil, clotrimazole and related compounds on the activity of microsomal epoxide hydrolase *in situ*, in purified form and in reconstituted systems towards different substrates.' *Eur. J. Biochem.*, 1986, **159**, 415–423.

183. M. O. James and K. B. Sloan, 'Structural features of imidazole derivatives that enhance styrene oxide hydrolase activity in rat hepatic microsomes.' *J. Med. Chem.*, 1985, **28**, 1120–1124.

184. T. M. Guenthner, 'Selective inhibition and selective induction of multiple microsomal epoxide hydrolases.' *Biochem. Pharmacol.*, 1986, **35**, 839–845.

185. R. A. Lubet, K. H. Dragnev, D. P. Chauhan *et al.*, 'A pleiotropic response to phenobarbital-type enzyme inducers in the F344 NCr rat. Effects of chemicals of varied structure.' *Biochem. Pharmacol.*, 1992, **43**, 1067–1078.

186. S. G. Kim, 'Transcriptional regulation of rat microsomal epoxide hydrolase gene by imidazole antimycotic agents.' *Mol. Pharmacol.*, 1992, **42**, 273–279.

187. S. G. Kim, K. H. Jung, W. K. Yang *et al.*, 'Differential expression of microsomal epoxide hydrolase gene by azole heterocycles in rats.' *Biochem. Pharmacol.*, 1994, **48**, 111–120.

188. N. D. Kim, S. G. Kim and M. K. Kwak, 'Enhanced expression of rat microsomal epoxide hydrolase gene by organosulfur compounds.' *Biochem. Pharmacol.*, 1994, **47**, 541–547.

189. S. G. Kim, J. Y. Kwak, J. W. Lee *et al.*, 'Malotilate, a hepatoprotectant, suppresses CYP2E1 expression in rats.' *Biochem. Biophys. Res. Commun.*, 1994, **200**, 1414–1420.

190. J. E. Sheehan, H. C. Pitot and C. B. Kasper, 'Transcriptional regulation and localization of the tissue-specific induction of epoxide hydrolase by lead acetate in rat kidney.' *J. Biol. Chem.*, 1991, **266**, 5122–5127.

191. S. Gill and S. Kaur, 'Hepatic epoxide hydrolase acitivities and their induction by clofibrate and diethylhexylphthalate in various strains of mice.' *Biochem. Pharmacol.*, 1987, **36**, 4221–4227.

192. D. E. Moody, K. A. Montgomery, M. B. Ashour *et al.*, 'Effects of environmentally encountered epoxides on mouse liver epoxide-metabolizing enzymes.' *Biochem. Pharmacol.*, 1991, **41**, 1625–1637.

193. E. K. Yang, A. Radominska, B. S. Winder *et al.*, 'Dietary lipids coinduce xenobiotic metabolizing enzymes in rat liver.' *Biochim. Biophys. Acta*, 1993, **1168**, 52–58.

194. B. Lundgren, J. Meijer and J. W. DePierre, 'Induction of cytosolic and microsomal epoxide hydrolases and proliferation of peroxisomes and mitochondria in mouse liver after dietary exposure to *p*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid.' *Biochem. Pharmacol.*, 1987, **36**, 815–821.

195. B. Lundgren and J. W. DePierre, 'Induction of xenobiotic-metabolizing enzymes and peroxisome proliferation in rat liver caused by dietary exposure to di(2-ethylhexyl)phosphate.' *Xenobiotica*, 1987, **17**, 585–593.

196. H. R. Glatt and F. Oesch, 'Species differences in enzymes controlling reactive epoxides.' *Arch. Toxicol. Suppl.*, 1987, **10**, 111–124.

197. A. Astrom, S. Maner and J. W. DePierre, 'Induction of liver microsomal epoxide hydrolase, UDP-glucuronyl transferase and cytosolic glutathione transferase in different rodent species by 2-acetylaminofluorene or 3-methylcholanthrene.' *Xenobiotica*, 1987, **17**, 155–163.

198. B. Schilter and C. J. Omiecinski, 'Regional distribution and expression modulation of cytochrome P450 and epoxide hydrolase mRNAs in the rat brain.' *Mol. Pharmacol.*, 1993, **44**, 990–996.

199. J. P. Hardwick, F. J. Gonzalez and C. B. Kasper, 'Transcriptional regulation of rat liver epoxide hydratase, NADPH-cytochrome P-450 oxido-reductase, and cytochrome P-450b genes by phenobarbital.' *J. Biol. Chem.*, 1983, **258**, 8081–8085.

200. J. A. Craft, N. J. Bulleid, M. R. Jackson *et al.*, 'Induction of microsomal epoxide hydrolase by nitrosamines in rat liver. Effect on messenger ribonucleic acids.' *Biochem. Pharmacol.*, 1988, **37**, 297–302.

201. D. E. Moody, G. A. Clawson, D. A. Geller *et al.*, 'Sodium cholate extraction of rat liver nuclear xenobiotic-metabolizing enzymes.' *Biochem. Pharmacol.*, 1988, **37**, 1331–1341.

202. D. L. Simmons, P. McQuiddy and C. B. Kasper, 'Induction of the hepatic mixed-function oxidase system by synthetic glucocorticoids. Transcriptional and post-transcriptional regulation.' *J. Biol. Chem.*, 1987, **262**, 326–332.

203. S. G. Kim and Y. H. Kim, 'Gender-related expression of rat microsomal epoxide hydrolase during maturation: post-transcriptional regulation.' *Mol. Pharmacol.*, 1992, **42**, 75–81.

204. R. N. Wixtrom, M. H. Silva and B. D. Hammock, 'Affinity purification of cytosolic epoxide hydrolase using derivatized epoxy-activated Sepharose gels.' *Anal. Biochem.*, 1988, **169**, 71–80.

205. R. N. Wixtrom, M. H. Silva and B. D. Hammock, 'Cytosolic epoxide hydrolase in human placenta.' *Placenta*, 1988, **9**, 559–563.

206. M. Arand, M. Knehr, H. Thomas *et al.*, 'An impaired peroxisomal targeting sequence leading to an unusual bicompartmental distribution of cytosolic epoxide hydrolase.' *FEBS Lett.*, 1991, **294**, 19–22.

207. S. J. Gill, D. F. Grant, J. K. Beetham *et al.*, in 'Peroxisome Proliferators: Unique Inducers of Drug-Metabolizing Enzymes,' ed. D. E. Moody, CRC Press, Boca Raton, FL, 1994, pp. 113–121.

208. M. Knehr, H. Thomas, M. Arand *et al.*, 'Isolation and characterization of a cDNA encoding rat liver cytosolic epoxide hydrolase and its functional expression in *Escherichia coli*.' *J. Biol. Chem.*, 1993, **268**, 17623–17627.

209. D. F. Grant, D. H. Storms and B. D. Hammock, 'Molecular cloning and expression of murine liver soluble epoxide hydrolase.' *J. Biol. Chem.*, 1993, **268**, 17628–17633.

210. J. K. Beetham, T. Tian and B. D. Hammock, 'cDNA cloning and expression of a soluble epoxide hydrolase from human liver.' *Arch. Biochem. Biophys.*, 1993, **305**, 197–201.

211. K. K. Norris, T. M. DeAngelo and E. S. Vesell, 'Genetic and environmental factors that regulate cytosolic epoxide hydrolase activity in normal human lymphocytes.' *J. Clin. Invest.*, 1989, **84**, 1749–1756.

212. D. F. Grant, J. L. Spearow, D. H. Storms *et al.*, 'Chromosomal mapping and expression levels of a mouse soluble epoxide hydrolase gene.' *Pharmacogenetics*, 1994, **4**, 64–72.

213. C. Larsson, I. White, C. Johansson *et al.*, 'Localization of the human soluble epoxide hydrolase gene (EPHX2) to chromosomal region 8p21-p12.' *Hum. Genet.*, 1995, **95**, 356–358.

214. B. D. Hammock, M. El-Tantawy, S. S. Gill *et al.*, in 'Microsomes, Drug Oxidations, and Chemical Carcinogenesis,' ed. M. J. Coon, Academic Press, New York, 1980, pp. 654–658.

215. B. Lundgren, J. Meijer, W. Birberg *et al.*, 'Induction of cytosolic and microsomal epoxide hydrolases in mouse liver by peroxisome proliferators, with special emphasis on structural analogues of 2-ethylhexanoic acid.' *Chem. Biol. Interact.*, 1988, **68**, 219–240.

216. D. E. Moody, D. N. Loury and B. D. Hammock, 'Epoxide metabolism in the liver of mice treated with clofibrate (ethyl-α-(*p*-chlorophenoxy)isobutyrate), a peroxisome proliferator.' *Toxicol. Appl. Pharmacol.*, 1985, **78**, 351–362.

217. D. E. Moody and B. D. Hammock, 'The effect of tridiphane (2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl)oxirane) on hepatic epoxide-metabolizing enzymes: indications of peroxisome proliferation.' *Toxicol. Appl. Pharmacol.*, 1987, **89**, 37–48.

218. H. Thomas, L. Schladt, M. Knehr *et al.*, 'Effect of hypolipidemic compounds on lauric acid hydroxylation and phase II enzymes.' *Biochem. Pharmacol.*, 1989, **38**, 1963–1969.

219. J. K. Reddy and N. D. Lalwai, 'Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans.' *Crit. Rev. Toxicol.*, 1983, **12**, 1–58.

220. B. Chatterjee, W. F. Demyan, N. D. Lalwani *et al.*, 'Reversible alteration of hepatic messenger RNA species for peroxisomal and non-peroxisomal proteins induced by the hypolipidaemic drug Wy-14643.' *Biochem. J.*, 1983, **214**, 879–883.

221. S. McQuaid, S. E. Russell, S. A. Withe *et al.*, 'Analysis of transcripts homologous to acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase induced in rat liver by methylclofenapate.' *Cancer Lett.*, 1987, **37**, 115–121.

222. J. K. Reddy, S. K. Goel, M. R. Nemali *et al.*, 'Transcription regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase 3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators.' *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 1747–1751.

223. M. Hijikata, J. K. Wen, T. Osumi *et al.*, 'Rat peroxisomal 3-ketoacyl-CoA thiolase gene. Occurrence of two closely related but differentially regulated genes.' *J. Biol. Chem.*, 1990, **265**, 4600–4606.

224. D. F. Grant, D. E. Moody, J. Beetham *et al.*, in 'Peroxisome Proliferators: Unique Inducers of Drug-Metabolizing Enzymes,' ed. D. E. Moody, CRC Press, Boca Raton, FL, 1994, pp. 97–112.

225. M. S. Rao and J. K. Reddy, 'An overview of peroxisome proliferator-induced hepatocarcinogenesis.' *Environ. Health Perspect.*, 1991, **93**, 205–209.

226. D. E. Moody, G. G. Gibson, D. F. Grant *et al.*, 'Peroxisome proliferators, a unique set of drug-metabolizing enzyme inducers: commentary on a symposium.' *Drug Metab. Dispos.*, 1992, **20**, 779–791.

227. M. Hamberg and G. Hamberg, 'Hydroperoxide-dependent epoxidation of unsaturated fatty acids in the broad bean (*Vicia faba L.*).' *Arch. Biochem. Biophys.*, 1990, **283**, 409–416.

228. A. Sevanian, J. F. Mead and R. F. Stein, in 'Molecular Aspects of Environmental Toxicology,' ed. R. S. Bhatnager, Ann Arbor Science, Ann Arbor, MI, 1980, pp. 213–228.

229. B. Lundgren and J. W. DePierre, 'Proliferation of peroxisomes and induction of cytosolic and microsomal epoxide hydrolases in different strains of mice and rats after dietary treatment with clofibrate.' *Xenobiotica*, 1989, **19**, 867–881.

230. D. N. Loury, D. E. Moody, B. W. Kim *et al.*, 'Effect of dietary clofibrate on epoxide hydrolase activity in tissues of mice.' *Biochem. Pharmacol.*, 1985, **34**, 1827–1833.

231. B. L. Finley and B. D. Hammock, 'Increased cholesterol epoxide hydrolase activity in clofibrate-fed animals.' *Biochem. Pharmacol.*, 1988, **37**, 3169–3175.

232. D. E. Moody, M. H. Silva and B. D. Hammock, 'Epoxide hydrolysis in the cytosol of rat liver, kidney, and testis. Measurement in the presence of glutathione and the effect of dietary clofibrate.' *Biochem. Pharmacol.*, 1986, **35**, 2073–2080.

233. L. Schladt, R. Hartmann, Č. Timms *et al.*, 'Concomitant induction of cytosolic but not microsomal epoxide hydrolase with peroxisomal beta-oxidation by various hypolipidemic compounds.' *Biochem. Pharmacol.*, 1987, **36**, 345–351.

234. M. J. Graham, S. A. Wilson, M. A. Winham *et al.*, 'Lack of peroxisome proliferation in marmoset liver following treatment with ciprofibrate for 3 years.' *Fundam. Appl. Toxicol.*, 1994, **22**, 58–64.

235. A. M. Eriksson, M. A. Zetterqvist, B. Lundgren *et al.*, 'Studies on the intracellular distributions of soluble epoxide hydrolase and of catalase by digitonin-permeabilization of hepatocytes isolated from control and clofibrate-treated mice.' *Eur. J. Biochem.*, 1991, **198**, 471–476.

236. D. I. Crane, J. Zamattia and C. J. Master, 'Alterations in the integrity of peroxisomal membranes in livers of mice treated with peroxisome proliferators.' *Mol. Cell. Biochem.*, 1990, **96**, 153–161.

237. M. Hollinshead and J. Meijer, 'Immunocytochemical analysis of soluble epoxide hydrolase and catalase in mouse and rat hepatocytes demonstrates a peroxisomal localization before and after clofibrate treatment.' *Eur. J. Cell Biol.*, 1988, **46**, 394–402.

238. E. C. Dietze, J. Magdalou and B. D. Hammock, 'Human and murine cytosolic epoxide hydrolase: physical and structural properties.' *Int. J. Biochem.*, 1990, **22**, 461–470.

239. J. Meijer and J. W. Depierre, 'Properties of cytosolic epoxide hydrolase purified from the liver of untreated and clofibrate-treated mice. Purification procedure and physicochemical characterization of the pure enzymes.' *Eur. J. Biochem.*, 1985, **148**, 421–430.

240. V. Joste and J. Meijer, '*In vitro* translation of cytosolic and peroxisomal epoxide hydrolase and catalase on liver polyribosomes from untreated and clofibrate-treated C57Bl 6 mice.' *FEBS Lett.*, 1989, **249**, 83–88.

241. T. Osumi, J. K. Wen and T. Hashimoto, 'Two *cis*-acting regulatory sequences in the peroxisome proliferator-responsive enhancer region of rat acyl-CoA oxidase gene.' *Biochem. Biophys. Res. Commun.*, 1991, **175**, 866–871.

242. A. S. Muerhoff, K. J. Griffin and E. F. Johnson, 'Characterization of a rabbit gene encoding a clofibrate-inducible fatty acid omega-hydroxylase: CYP4A6.' *Arch. Biochem. Biophys.*, 1992, **296**, 66.

243. J. Meijer and J. W. DePierre, 'Cytosolic epoxide hydrolase.' *Chem. Biol. Interact.*, 1988, **64**, 207–249.

244. J. Meijer, A. Bergstrand and J. W. DePierre, 'Preparation and characterization of subcellular fractions from the liver of C57Bl 6 mice, with special emphasis on their suitability for use in studies of epoxide hydrolase activities.' *Biochem. Pharmacol.*, 1987, **36**, 1139–1151.

245. C. L. Denlinger and E. S. Vesell, 'Hormonal regulation of the developmental pattern of epoxide hydrolases. Studies in rat liver.' *Biochem. Pharmacol.*, 1989, **38**, 603–610.

246. F. Pinot, D. F. Grant, J. F. Spearow *et al.*, 'Differential regulation of soluble epoxide hydrolase by clofibrate and sexual hormones in the liver and kidneys of mice.' *Biochem. Pharmacol.*, 1995, **50**, 501–508.