Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
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12 Mammalian Xenobiotic Epoxide Hydrolases

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General characteristics

FUNCTION

Epoxide hydrolases (EH, E.C.3.3.2.3) hydrolyse oxiranes, a specific class of cyclic ethers (Oesch 1973). The common feature of these compounds, their three-membered ring system, is under high tension, due to the unusually small bonding angles. Together with the polarisation of the C–O bonds, this leads to an enhanced chemical reactivity that is further modulated by the substitution pattern at the epoxide ring. Epoxides act as electrophiles with the reactive centre being one of the ring carbon atoms. As a general rule, asymmetric substitution at the ring enhances the reactivity. Important targets for epoxides in living organisms are nucleophilic sites in biomacromolecules, in particular proteins and nucleic acids. Chemical attack of these leads to cytotoxic and genotoxic effects. In particular, the modification of DNA bases can result in inheritable changes and such changes may ultimately give rise to carcinogenesis (Miller and Miller 1981). The primary function of xenobiotic epoxide hydrolases is to defeat such hazardous effects of epoxides. Epoxides can enter the body pre-formed or may arise from the metabolism of xenobiotic and, in some cases, of endogenous compounds (Figure 12.1).

In contrast to the great number of enzymes that can metabolise arenes or alkenes to epoxides, there are at present only two distinct mammalian xenobiotic epoxide hydrolases known (Oesch and Bentley 1976; Ota and Hammock 1980; Guenthner *et al.* 1981; Thomas *et al.* 1990; Hammock *et al.* 1997; Armstrong 1999). These two, the membrane-bound microsomal epoxide hydrolase (mEH) and the soluble epoxide hydrolase (sEH), will be described in detail in this chapter. Three more EHs have been identified in mammals that all have a narrow substrate specificity for epoxides formed from endogenous precursors, namely the leukotriene A4 hydrolase (Haeggstrom *et al.* 1990), the cholesterol epoxide hydrolase (Levin *et al.* 1983; Oesch *et al.* 1984) and

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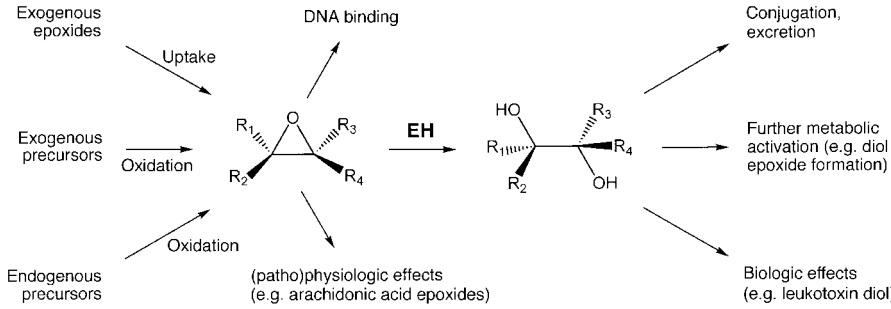


Figure 12.1 Role of epoxide hydrolases in the metabolism of exogenous and endogenous epoxides. EH: epoxide hydrolase.

the hepoxilin epoxide hydrolase (Pace-Asciak and Lee 1989). These will not be discussed here, since they have little, if any, impact on xenobiotic metabolism.

In general, xenobiotic epoxide hydrolases serve the above-described detoxification function, yet, as always, there are some exceptions to this rule (Bentley *et al.* 1977). A prominent example for this is the metabolic activation of polycyclic aromatic hydrocarbons (PAH) to the corresponding dihydrodiol epoxides, the ultimate carcinogenic metabolites of this class of compounds (Holder *et al.* 1974; Sims *et al.* 1974) (Figure 12.2). PAH with a so-called bay region are metabolically activated in a first step by, e.g. CYP (cytochrome P450), to pre-bay epoxides. These genotoxic metabolites can rapidly rearrange to the corresponding, much less toxic, phenols and thus undergo spontaneous detoxification. Likewise, enzymic cleavage by epoxide hydrolases to the corresponding vicinal dihydrodiols leads to *per se* inactive products. However, these diols are again substrates for a variety of CYP and COX isoenzymes which finally generate the highly reactive dihydrodiol epoxides. These compounds neither undergo rearrangement to phenols (they are alkene, not arene oxides), nor are they substrates (or in some cases only extremely poor substrates) for epoxide hydrolases. Their detoxification by glutathione conjugation (Jernstrom *et al.* 1992), the last line of defence, is obviously not sufficient to protect the organism from the potent carcinogenic effect of these metabolites. The central importance of mEH for this activation pathway has finally been proven using mEH *knockout* mice (Miyata *et al.* 1999). In contrast to their wild-type relatives, these animals were highly resistant to the carcinogenic effect of 7,12-dimethylbenz[*a*]anthracene in the mouse skin tumorigenesis test.

A second, recently discovered and somehow unexpected activation pathway driven by epoxide hydrolases is the formation of toxic vicinal diols from the epoxides of unsaturated fatty acids. Leukotoxin, the epoxide of linolenic acid, has earlier been reported to be the chemical mediator in multiple organ failure and adult respiratory distress syndrom (ARDS) (Ozawa *et al.* 1991). It now appears that the diol rather than the epoxide seems to be the causative agent (Moghaddam *et al.* 1997): in mice, sEH is the major leukotoxin-metabolising EH. Pretreatment of mice with an sEH inhibitor significantly increased the tolerance of the animals to the toxic effects of leukotoxin.

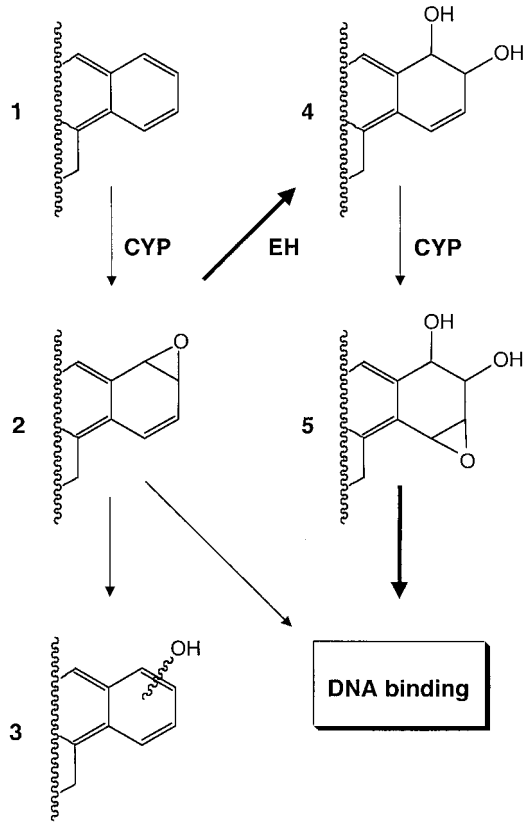


Figure 12.2 Role of epoxide hydrolase in the metabolic activation of polycyclic aromatic hydrocarbons (PAH) to diol epoxides.

1 = parent PAH; 2 = PAH pre-bay epoxide; 3 = PAH phenol (two regioisomers possible); 4 = PAH dihydrodiol; 5 = PAH dihydrodiol epoxide. CYP, cytochrome P_{450}: EH, epoxide hydrolase.

Likewise, recombinant expression of sEH enhanced leukotoxin cytotoxicity in a number of different cell systems.

In summary, the vast majority of substrates are chemically inactivated and thus detoxified by EH but in some specific cases, epoxide hydrolysis can directly or indirectly increase the toxicity of the respective substrate.

PHYLOGENETICS

The mEH was among the first xenobiotic metabolising enzymes to be cloned (Gonzalez and Kasper 1981) and characterised in terms of amino acid sequence (Heinemann and Ozols 1984), yet little immediate progress resulted from these early findings. At the end of the 1980s, a bacterial enzyme with marginal sequence

similarity to the mEH was discovered, a haloalkane dehalogenase (Janssen *et al*. 1989), but this possible relationship was largely ignored. This changed dramatically when the molecular characterisation of sEH was reported (Beetham *et al*. 1993; Grant *et al*. 1993; Knehr *et al*. 1993). At that time, direct comparison of the two epoxide hydrolase sequences did not show any convincing relationship between the two, yet the sEH, like the mEH before, showed marginal but significant similarity to the bacterial haloalkane dehalogenase (Arand *et al*. 1994), of which the three-dimensional structure had just been determined (Franken *et al*. 1991; Verschueren *et al*. 1993). The dehalogenase had been identified as a member of the α/β hydrolase fold family of enzymes (Ollis *et al*. 1992), its most famous relative thus being the acetylcholine esterase. The fact that the overall protein fold is conserved in this family of enzymes, despite the lack of evident sequence similarity, strongly suggested that proteins related to the dehalogenase by sequence similarity should have the same three-dimensional structure, and therefore EHs should also be members of the α/β hydrolase fold enzyme family (Arand *et al*. 1994; Lacourciere and Armstrong 1994; Pries *et al*. 1994). Final proof for this has recently been provided by X-ray analysis of a variety of EH structures (Argiriadi *et al*. 1999; Nardini *et al*. 1999; Zou *et al*. 2000). The generic EH structure derived from this is the following (Figure 12.3).

The central domain of EH is the α/β hydrolase fold that is composed of a central β-

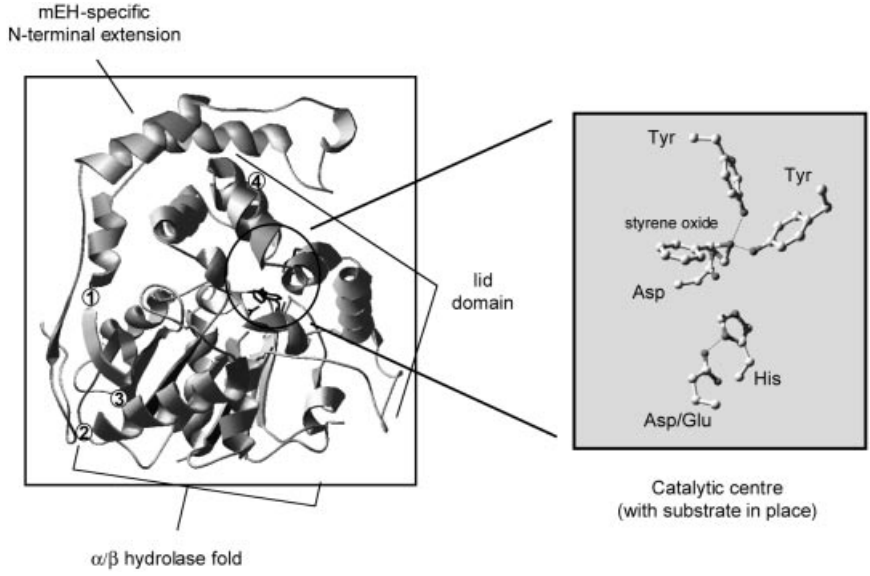


Figure 12.3 Three-dimensional structure of epoxide hydrolases.

The left part of the figure shows a ribbon model of the EH structure while the right side shows a magnification of the active centre, with styrene oxide as a generic EH substrate in place. Note that the *N*-terminal extension is a unique feature of the mEH-like epoxide hydrolases. The numbered circles in the ribbon representation denote the position of the following individual polymorphisms of EHs that are discussed later in this chapter. $1 = Tyr_{113}His$ (mEH); $2 = Arg_{139}His$ (mEH); $3 = Arg_{287}Gln$ (sEH); $4 = Arg_{402}Arg/Arg$ (insertion;sEH).

sheet flanked by α-helices. On top of this fold sits a so-called lid. The catalytic site is situated at the interface of these two structural entities, with a catalytic triad (Ollis *et al.* 1992) being anchored in the α/β hydrolase fold and two catalytic tyrosines hanging from the lid into the substrate binding pocket. One very important aspect of this discovery was its impact on the understanding of the catalytic mechanism of enzymic epoxide hydrolysis, (see below). Furthermore, the comparison between the EHs and the dehalogenase revealed a number of typical signature sequences that define a subgroup in the family of α/β hydrolase fold enzymes. These signatures can be used to scan the available biological databases for the identification of other potential epoxide hydrolases. A phylogenetic tree of a selection of sequences retrieved this way is shown in Figure 12.4.

The first lesson to be learned from this multiple-sequence comparison is that mEH

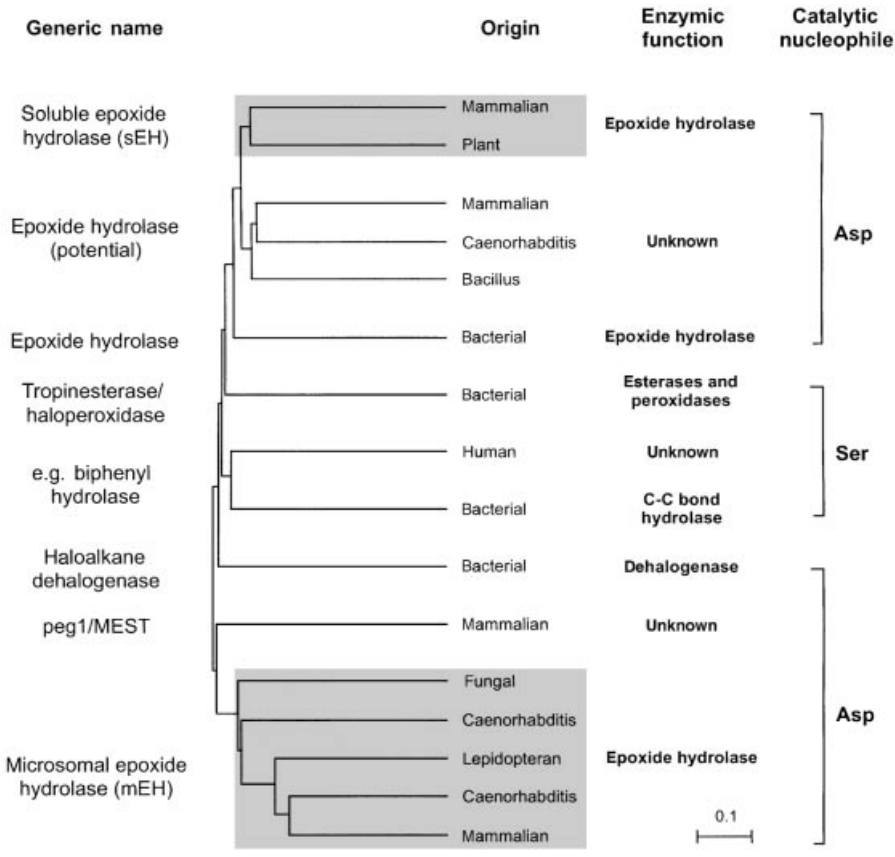


Figure 12.4 Phylogenetic tree of epoxide hydrolase/haloalkane dehalogenase-related α/β hydrolase fold enzymes.

Comparison has been performed using CLUSTAL-X (Thompson *et al.* 1997). For further details of the analysis see Arand *et al.* (1999a).

and sEH are, indeed, at the opposite ends of this comparison, and they must have evolved from their common ancestor several billion years ago. The mammalian mEH has apparent orthologues in insects (Wojtasek and Prestwich 1996), nematodes (Wilson *et al.* 1994) and in fungi (Arand *et al.* 1999a), while sEH orthologous enzymes have been identified in plants (Kiyosue *et al.* 1994; Stapleton *et al.* 1994). A second finding is the broad variety of different enzymes in this family tree, ranging from epoxide hydrolases over esterases to C–C bond hydrolases. As will be detailed later, mammalian xenobiotic epoxide hydrolases have an aspartic acid residue in their active site serving as the catalytic nucleophile. Unexpectedly, a number of enzymes in the alignment revealed a serine in this position, namely the esterases and C–C bond hydrolases, and this results in a dilemma in terms of enzyme nomenclature: while these serine-nucleophile enzymes are functionally related to esterases, they are included in the epoxide hydrolase/dehalogenase-like α/β hydrolase fold enzymes on the basis of their structure, and it will be difficult to establish a widely accepted nomenclature system as is now available for CYP (Nelson *et al.* 1996), UGT (Mackenzie *et al.* 1997) and GST (Hayes and Pulford 1995) enzymes. So far, it has been proposed to name mEH HYL1, mammalian sEH HYL2 and plant sEH HYL3 (Beetham *et al.* 1995), yet this attempt must be regarded as preliminary since the other enzymes related to EH should be incorporated into this nomenclature system.

MECHANISM

The mechanism of enzymic epoxide hydrolysis has been subject of intense investigation since the 1970s (DuBois *et al.* 1978), and its understanding offers a clue to the incredible yet hidden efficacy of EHs. It was a kind of mystery how a single enzyme, the mEH, could—on the one hand—have an enormously broad substrate specificity while—on the other—displaying an apparently high affinity to different substrates, sufficient to detoxify these at low concentrations.

The first indication of an unusual mode of action was provided by the notion that a single round of substrate turnover in the presence of heavy water led to the incorporation of ^{18}O into the enzyme rather than into the reaction product (Lacourciere and Armstrong 1993), an observation incompatible with the previously favoured direct hydrolysis of epoxides by EH (Armstrong 1987). The authors reasoned that the formation of an enzyme-substrate ester intermediate must have taken place, a deduction that was further substantiated by the above-described sequence comparison (Arand *et al.* 1994) between EH and other enzymes for which the formation of similar ester intermediates in the course of their enzymic reaction had already been shown. Biochemical (Pinot *et al.* 1995; Arand *et al.* 1996, 1999b; Rink *et al.* 1997, 2000; Laughlin *et al.* 1998; Tzeng *et al.* 1998; Yamada *et al.* 2000) and structural (Argiriadi *et al.* 1999; Nardini *et al.* 1999; Zou *et al.* 2000) analyses of EHs then led to a detailed understanding of the process (Figure 12.5).

The key event in the initial substrate recognition of epoxides by EH seems to be the trapping of the epoxide oxygen by two tyrosine residues via hydrogen bonding in the active site of the enzyme. Also contributing to this initial, reversible binding may be some hydrophobic interactions between the lipophilic side chain of the substrate and the surface of the substrate access tunnel of the enzyme. Here, also, some constraints

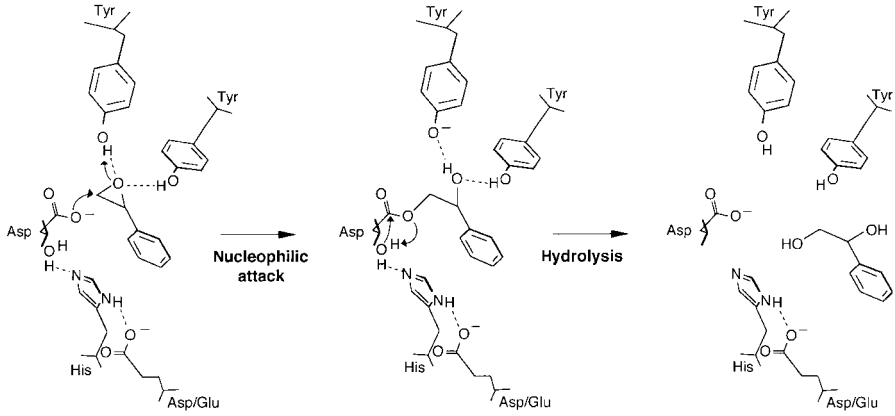


Figure 12.5 Catalytic mechanism of enzymic epoxide hydrolysis.

do apply. Due to the position of the active site residues at the end of its substrate access tunnel (Zou *et al*. 2000), mEH may be unable to hydrolyse *trans*-substituted epoxides, while the sEH, with its active site residues sitting on the side of a bent narrow tunnel (Argiriadi *et al*. 1999), does interact with these *trans*-substituted epoxides but cannot breakdown particularly bulky substrates.

The first chemical reaction step is the crucial one for substrate inactivation: the hydrogen bonding of the epoxide oxygen positions a ring carbon favourable for nucleophilic attack by the catalytic nucleophile of the EH catalytic triad, an aspartic acid residue. In a push–pull mechanism, this aspartic acid forms an ester bond with the ring carbon under scission of the respective C–O bond in the epoxide ring. Simultaneously, the oxygen is saturated by a proton from one of the two tyrosines. The resulting enzyme-substrate ester intermediate is subsequently hydrolysed by the water-activating charge relay system of the catalytic triad, composed of a histidine and an acidic residue, an aspartic acid in the case of sEH and a glutamic acid in the case of mEH.

An important observation was that the first step of this reaction, the ester formation, proceeds by orders of magnitudes faster than the second, hydrolytic step (Tzeng *et al*. 1996). First, this explains a likely underestimation of the detoxication efficacy of EH as illustrated in Figure 12.6, if the product formation is used as the measure for this. Second, it explains the apparent contradiction between broad substrate specificity and high substrate affinity. The relationship between the real affinity of the substrate to the enzyme, characterised by the dissociation constant K_D, and the apparent affinity measured as the Michaelis–Menten constant K_M is dependent on the rate constants of the nucleophilic attack (k_1) and the hydrolysis (k_2) as follows:

$$K_M = K_D \times \frac{k_2}{k_1 + k_2} \tag{12.1}$$

If, as in the present case, k_1 is orders of magnitudes higher than k_2, this equation essentially reduces to

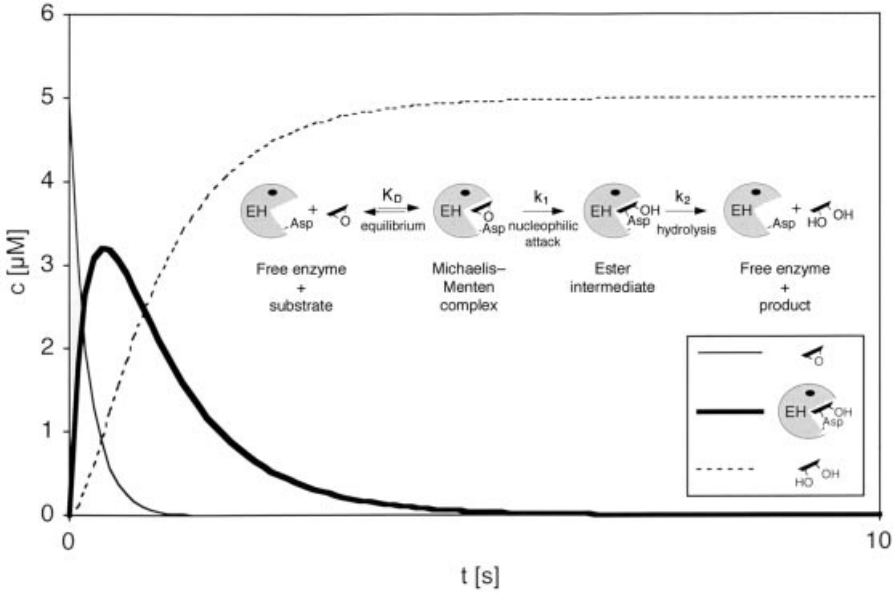


Figure 12.6 Detoxification kinetics of enzymic epoxide hydrolysis.

Displayed is the result of a computer simulation of styrene epoxide hydrolysis by human mEH. The concentrations of epoxide (narrow line), diol (broken line) and ester intermediate (bold line) are plotted over time. Under the chosen conditions (enzyme in excess over its substrate, as is probably true for mEH in most real life settings), the decline of the substrate concentration (i.e. of the toxic challenge) proceeds significantly faster than the increase in product formation, due to the intermediate accumulation of the enzyme-substrate ester. Thus, considering rate of detoxification by taking the initial rate of product formation as the measure results in a strong underestimation of the efficacy of the enzyme. For further discussion see Oesch *et al.* (2000).

$$K_M = K_D \times \frac{k_2}{k_1} \tag{12.2}$$

which can be further transformed to

$$\frac{K_M}{K_D} = \frac{k_2}{k_1} \tag{12.3}$$

Thus, the apparent high affinity of mEH for many substrates is actually based on a comparatively low real affinity and reflects the great difference between k_1 and k_2.

From the above it is evident that the enzymic epoxide hydrolysis is not optimised for product release. EH turnover rates are in the order of a few substrate molecules per second at best (Thomas *et al.* 1990). In contrast, acetylcholine esterase, a structural and functional relative (see above), achieves a turnover rate of 25,000 s^{-1}, despite the fact that the second step for both enzymic reactions is chemically practically the same, i.e. hydrolysis of the ester intermediate. We speculate that optimisation of the hydrolytic step could not be more successful in the case of EHs, since the ester intermediates of different substrates may have different spatial location in the active

site. We suggest that the epoxide side chain will put some constraint on the position of the attacked epoxide ring carbon which can most likely be compensated by an inferred flexibility of the aspartic acid that acts as the nucleophile. Since ester formation is the detoxification step, selection pressure is in favour to speed up this step rather than the subsequent hydrolysis. Indeed, it was possible to increase k_2 of mEH for a variety of different substrates by introducing a single-point mutation (Arand *et al.* 1999b), but the consequences for the turnover of other substrates have not been thoroughly investigated.

Microsomal epoxide hydrolase

STRUCTURAL CHARACTERISTICS

Mammalian mEH has a molecular mass of 51 kDa, corresponding to 455 amino acid residues (Porter *et al.* 1986). It is attached to the ER membrane by a single *N*-terminal membrane anchor (Friedberg *et al.* 1994). This anchor is connected to the generic α/β hydrolase fold by a stretch of about 100 amino acid residues that wraps around the molecule in a single large meander (see Figure 12.3), thereby apparently clamping together the α/β hydrolase fold and the lid on its top (Zou *et al.* 2000). This very compact structure may be a reason for an observed comparatively high resistance of mEH against thermal inactivation and proteolytic digestion. The quaternary structure of mammalian mEH is presently unknown. It has been speculated that the enzyme associates with the CYP and CYP reductase to a multienzyme complex, yet the few experimental approaches to prove this have not been conclusive (Oesch and Daly 1972; Etter *et al.* 1991). Since EH substrates are, in general, highly lipophilic, it is conceivable that the entry to the EH active site is directly connected to the lipid bilayer of the membrane, so that lipophilic compounds that would travel along the lipid phase could directly enter into it. A similar topology has just been reported for the xenobiotic-metabolising CYP2C5 (Williams *et al.* 2000). Such a scenario, with the two-dimensional membrane being the universal and efficient adaptor, would facilitate the interaction between EH and any CYP, without the need of direct interaction between the proteins.

After solubilisation from the membrane with detergents, EH is in the state of a homo-oligomer of an apparent molecular weight of 700-800 kDa (Guengerich and Davidson 1982). A soluble enzyme related to the mammalian mEH has been identified in *Aspergillus niger* (Morisseau *et al.* 1999a). This enzyme has been cloned (Arand *et al.* 1999a) and crystallised (Zou *et al.* 2000), and proved to be a homodimer in solution and in crystal form. The interaction surface between the two subunits involves the lid and the *N*-terminal meander and is apparently well conserved between fungal and mammalian enzymes. Thus, mEH may also exist as a homodimer in the membrane, possibly with its active site bent towards the lipid bilayer.

METABOLIC FUNCTION

Microsomal epoxide hydrolase is believed to be the major xenobiotic metabolising EH (Armstrong 1987). It has an extremely broad range of substrates, examples of which

are shown in Figure 12.7. In general, an mEH substrate should be an epoxide (so far, no exceptions have been identified), should be hydrophobic in nature and should be mono-,1,1-di- or 1,2-*cis*-disubstituted.

Bulky substrates, such as benzo[*a*]pyrene-4,5-oxide are as well accepted as slim compounds, e.g. octene-1,2-oxide. Since epoxides are potentially hazardous, not very many therapeutically used drugs undergo this metabolic pathway and thus, implications of mEH in clinical drug metabolism are, from a quantitative standpoint, not so numerous as those associated with CYP, glucunonide and sulphate conjugating enzymes, yet toxicologically especially important. One example is the anticonvulsant carbamazepine, a major metabolite of which is the 10,11-oxide (Eichelbaum *et al.* 1979). This symmetric epoxide is not very reactive and did not act as a mutagen in the Ames test (Glatt *et al.* 1983). Nevertheless, it has been suggested to be the cause of the adverse drug effects of carbamazepine after *in vivo* inhibition of mEH by co-medication with valpromide (see below) (Meijer *et al.* 1984).

Some industrial compounds are metabolically activated to epoxides. A prominent case is styrene, of which more than 90% of a given dose is converted to the 7,8-epoxide in the human body (Jenkins Sumner and Fennell 1994). It is at the same time an impressive example of the detoxification efficacy of mEH. Despite the fact that styrene oxide is a proven carcinogen (Ponomarkov *et al.* 1984), styrene itself is orders

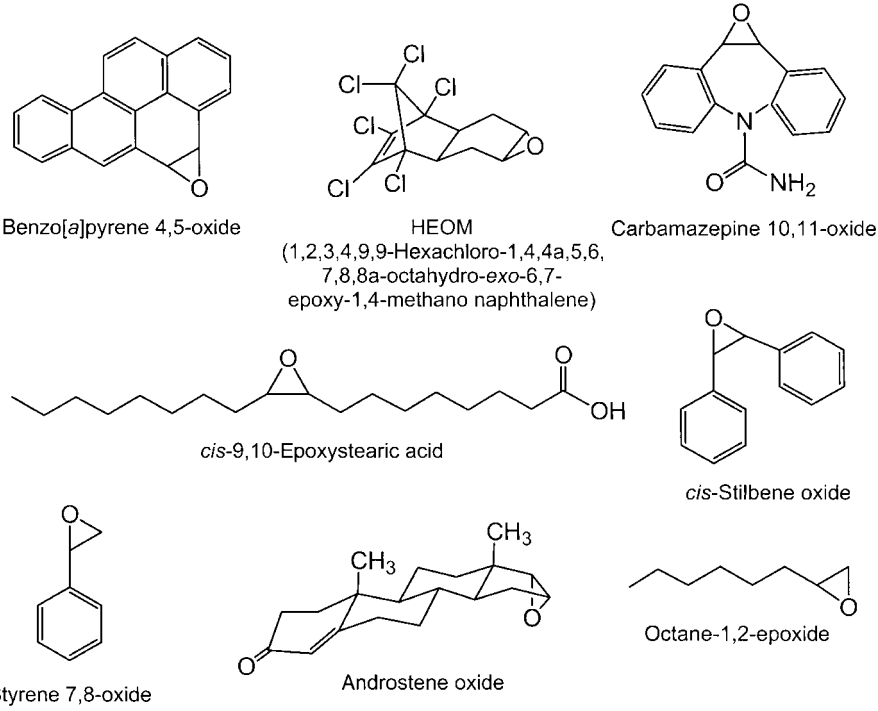


Figure 12.7 Typical substrates for mammalian mEH.

of magnitudes less harmful since the metabolically formed styrene-7,8-oxide is almost immediately hydrolysed by mEH, probably because the liver is the major site for both, formation as well as breakdown of the epoxide. Thus, it has been observed in styrene-exposed workers that the biomarkers of exposure to the reactive metabolite styrene oxide hardly correlate with the level of styrene exposure, but show significant correlation with exposure to exogenous styrene oxide that was present in roughly 1,000-fold lower concentration in the ambient air as compared to the styrene (Rappaport *et al*. 1996). This is in line with the observation that recombinant cell lines that express human mEH at a level comparable to that observed in human liver can tolerate up to a definable threshold a high concentration of styrene, without showing detectable signs of genotoxic damage (Herrero *et al*. 1997).

A number of possible endogenous functions have been attributed to mEH, the significance of which is not perfectly clear:

(1) The formation of 16α,17α-epoxides first from oestradiol (Breuer and Knuppen 1961) and later from androsterone was reported (Disse *et al*. 1980), and it was found thereafter that these were good substrates for mEH (Vogel-Bindel *et al*. 1982). Furthermore, the adrenal gland was reported to contain exceptionally high amounts of mEH (Papadopoulos *et al*. 1994). Very recently, high expression of mEH was observed in the corpus luteum, and a decrease of oestradiol production was observed on treatment with the mEH inhibitor 1,1,1-trichloro-2,3-propene oxide under conditions where the aromatase activity remained unaffected (Hattori *et al*. 2000). Finally, mEH has been identified as one component of a so-called anti-oestrogen binding site (AEBS) (Mesange *et al*. 1998).
(2) Similarly, mEH has been proposed to be a component of the vitamin K epoxide reductase (VKOR) (Guenthner *et al*. 1998).
(3) A highly controversial issue that splits the EH community in believers and disbelievers is its possible role in the membrane transport of bile acids (Alves *et al*. 1993), which is discussed in the context of subcellular localisation of the enzyme in the next section.

SUBCELLULAR LOCALISATION

The major location of mEH within the cell is the ER membrane. The above-mentioned bile acid carrier function implies a location of mEH on the plasma membrane that has, indeed, been claimed in a few reports (Alves *et al*. 1993; von Dippe *et al*. 1993, 1996; Zhu *et al*. 1999). However, many attempts by other researchers, including ourselves, to reproduce these findings have reportedly failed (Waechter *et al*. 1982; Craft *et al*. 1990; Honscha *et al*. 1995; Friedberg *et al*. 1996; Holler *et al*. 1997), and we, therefore, find the present proof for the mEH being a genuine plasma membrane constituent not unambiguously conclusive. The topology of mEH within the ER membrane has been a subject of intense research for some time (Porter *et al*. 1986; Craft *et al*. 1990). It finally turned out that mEH is attached to the membrane with a single *N*-terminal anchor (Friedberg *et al*. 1994) and that at least the mammalian enzyme is oriented towards the cell cytosol (Holler *et al*. 1997), as are the CYP

enzymes, and not towards the ER lumen, as are the UGTs (glucuronosyl transferases; Figure 12.8).

TISSUE DISTRIBUTION

Early studies have identified EH activity in almost every tissue that was analysed, which led to the statement that mEH is apparently ubiquitously expressed in rat organs (Oesch *et al*. 1977a). These findings, referring to the distribution of enzyme activity among organs, were later refined to tissue compartments and cell types, using a variety of techniques such as immunohistochemistry or cell sorting (Bentley *et al*. 1979; Wolf *et al*. 1984; Guenthner and Karnezis 1986; Steinberg *et al*. 1987; Bogdanffy 1990; Farin and Omiecinski 1993; Backman *et al*. 1999; Hattori *et al*. 2000; Kessler *et al*. 2000). Indeed, a large variety of cell types express mEH to appreciable levels, but in many others mEH expression is below the level of detection. This should be borne in mind when talking about the apparently ubiquitous mEH expression. Expression of mEH is usually highest in the liver, followed by testes, adrenal gland, lung, kidney and intestine (in the mouse, interestingly, higher in testis than in the liver) (Thomas *et al*. 1990; Hammock *et al*. 1997). However, this order may vary from species to species. In humans, for instance, a particularly high mEH content has been reported for the adrenal gland (Papadopoulos *et al*. 1994). In rats, three different transcripts for mEH have been described, that are divergent in their 5′-non-coding region but code for identical polypeptides (Honscha *et al*. 1991). It was concluded, that at least three alternative non-coding first exons exist in the rat mEH gene which would allow for three independant regions of transcriptional control. Five such non-coding first exons

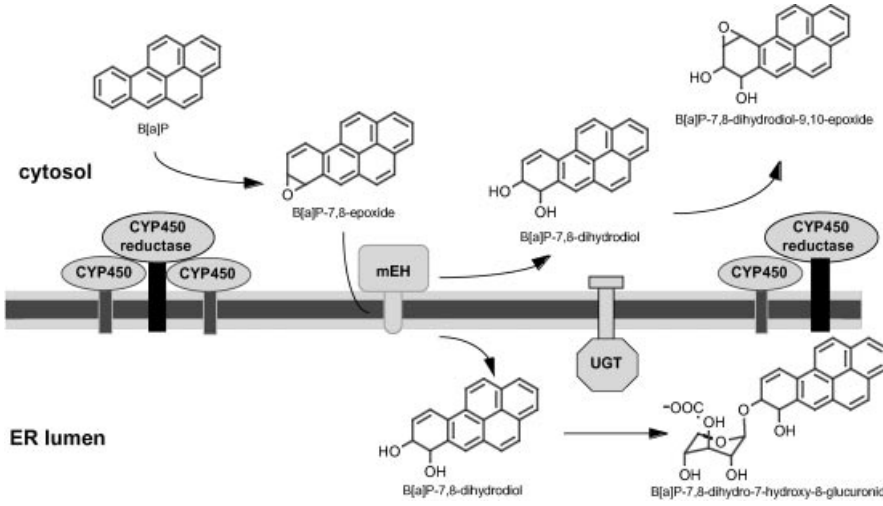


Figure 12.8 Membrane topology of mEH in comparison to other ER-resident xenobiotic-metabolising enzymes.

Part of the metabolism of benzo[*a*]pyrene is incorporated into the figure as an example of the metabolic cooperation of the different enzymes.

have recently been described for the human mEH gene and their tissue-specific expression was reported (Gaedigk *et al*. 1997). Thus, one reason for the wide distribution of mEH in mammalian tissues is obviously due to the existence of multiple promoters in the mEH gene.

SPECIES DIFFERENCES

Marked species differences in the expression level of mEH between mammals exist. Under certain circumstances, these may be crucial for differential toxic effects in different species (Oesch *et al*. 1977b). Of the classic laboratory animals, the mouse is low in mEH, with about 0.1–0.2% of the microsomal protein being mEH. In contrast, human liver mEH constitutes well above 1% of the microsomal protein, while in the rat it is between 0.5% and 1%. This is possibly one reason why high doses of styrene, above a threshold of about 300 ppm in the ambient air, led to a strong increase in styrene oxide blood levels in mice while this was not observed with rats under similar conditions (Kessler *et al*. 1992).

INDUCIBILITY BY FOREIGN COMPOUNDS

Microsomal EH is—despite its already high concentration in liver—inducible by a large variety of different compounds in laboratory animals. In view of the above-described enzymic mechanism this is beneficial even if the enzyme is already in apparent excess over its substrate, because the steady-state level of its substrates is, in any case, inversely correlated with the mEH concentration. 2-Acetylaminofluorene (Astrom and DePierre 1981) and *trans*-stilbene oxide (Schmassmann and Oesch 1978) are among the most potent inducers, leading to up to a 7-fold increase of enzyme activity in rat liver. Other inducers include phenobarbital (Oesch *et al*. 1971a), imidazole derivatives (Kim *et al*. 1995), lead acetate (Sheehan *et al*. 1991) and peroxisome proliferators (Oesch and Arand 1994). The existence of several independent promoters in the mEH gene (see above) that are differentially regulated complicates the analysis of transcriptional regulation since the different inducers will most likely act on different transcriptional units of the gene.

INHIBITORS

The first mEH inhibitors that were identified (Oesch *et al*. 1971b) can, on the basis of current understanding, all be regarded as substrates with a low k_2, i.e. a low K_M and a low V_{max} (see above). Of these, 1,1,1-trichloro-2,3-propene oxide (TCPO) (Figure 12.9) has been the most widely used. Later, valpromide was identified as the first non-substrate inhibitor of mEH, on the basis of its interference with the carbamazepine metabolism (see above) (Meijer *et al*. 1984). The amide group seems to mimic the epoxide ring, most likely in that the amide carbonyl hydrogen bonds to the tyrosines while the amino group hydrogen bonds to the nucleophilic aspartate. The hydrophobic side chain of valpromide is obviously well suited to fit the substrate access tunnel. Obviously, valpromide has a much lower K_D with mEH than most of the substrates, thus making up for the lack of covalent binding to the enzyme. The advantage of using

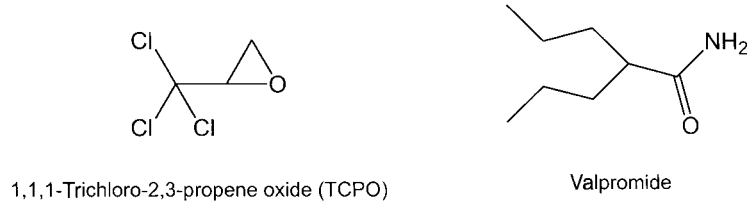


Figure 12.9 Prototypes of mEH inhibitors.

a non-substrate inhibitor is obvious: as long as the inhibitor is itself a substrate for the enzyme it can be consumed over time and thus lose its inhibitory potency. Indeed, TCPO is no safe inhibitor if used over a longer period of time in the presence of substantial amounts of mEH.

GENETIC POLYMORPHISMS

Two genetic polymorphisms affecting the primary sequence of the mEH protein in humans have been described (Hassett *et al*. 1994), and quite a number of epidemiological studies has monitored the prevalence of the different alleles in different subgroups of the population, especially with respect to disease susceptibility (McGlynn *et al*. 1995; Lancaster *et al*. 1996; Benhamou *et al*. 1998). The two polymorphisms represent single amino acid exchanges, namely $Tyr_{113}His$ or $Arg_{139}His$, none of which apparently affects the enzyme kinetics or substrate selectivity. This is not surprising, since both polymorphic sites lie on the surface of the protein (see Figure 12.3), far away from the catalytic centre. However, both polymorphisms appear to moderately affect the protein stability, resulting—at best—in a 2-fold difference in the enzyme tissue concentration. This is far from the observed maximum interindividual difference in enzymic activity reported in human liver (Mertes *et al*. 1985; Hassett *et al*. 1997), and thus is unlikely to be a major contributor to this variability. Likewise, polymorphisms (single nucleotide polymorphisms; SNPs) in the promoter region of the human mEH gene have been identified (Raaka *et al*. 1998) that may have a minor influence ($\pm$30%) on the transcription efficacy of the gene, which is, at best, a minor contribution to overall variability. However, since only one of the at least five promoter regions of the human gene (see above) has been addressed in this study, there is a good chance that more relevant polymorphisms in the regulatory regions of the gene await detection.

Soluble epoxide hydrolase

STRUCTURAL CHARACTERISTICS

Mammalian sEH is a homodimer in solution, with a subunit molecular mass of 62 kDa, corresponding to 554 amino acid residues (Beetham *et al*. 1993; Grant *et al*. 1993; Knehr *et al*. 1993). The generic EH α/β hydrolase fold is built by the *C*-terminal 320 amino acid residues while the *N*-terminal 220 amino acid residues comprise a

second domain, harbouring a potential second catalytic site, the function of which is as yet unknown. From X-ray analysis of mouse sEH (Argiriadi *et al.* 1999), one important function deduced for the *N*-terminal domain was to stabilise the overall structure, since the *N*-terminal domain of subunit A largely interacts with the *C*-terminal domain of subunit B and vice versa.

METABOLIC FUNCTION

The soluble epoxide hydrolase complements the mEH in the metabolism of xenobiotic epoxides in that it is capable of hydrolysing 1,2-*trans*-substituted oxiranes (Ota and Hammock 1980). Typical examples of this group of compounds are *trans*-stilbene oxide and *trans*-ethylstyrene oxide. Treatment with the latter compound leads to sister chromatide exchange in human lymphocytes (Krämer *et al.* 1991). In these cells the individual susceptibility to this is negatively correlated with the expression level of sEH. As a general rule, bulky substrates are not accepted, but a limited number of PAH epoxides are converted to diols by sEH (Figure 12.10) (Oesch and Golan 1980). Nevertheless, the clear domain of sEH are epoxides derived from fatty acids. Substrates within this group range from arachidonic acid epoxides (Zeldin *et al.* 1993) over

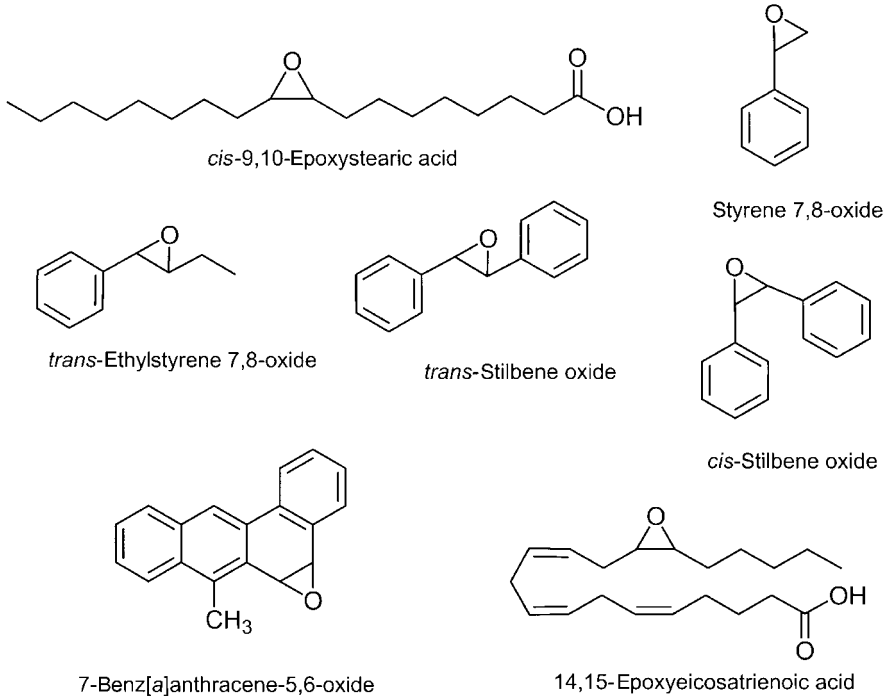


Figure 12.10 Typical substrates for mammalian sEH.

leukotoxin (Moghaddam *et al*. 1997) to diepoxides (Nourooz-Zadeh *et al*. 1992). The observation that some of these substrates and/or their corresponding diols apparently have (patho)physiological functions points towards a major endogenous role for sEH. In line with this, the sEH knockout mouse has a reduced blood pressure, indicating a modulating function of sEH in blood pressure regulation (Sinal *et al*. 2000).

A particularly interesting aspect of sEH is the potential second catalytic centre. Sequence similarity analysis has grouped the *N*-terminal domain into a large family of hydrolytic enzymes, including dehalogenases (different from the haloalkane dehalogenase) and phosphatases (Koonin and Tatusov 1994). The fact that a putative substrate binding cavity as well as the catalytic residues are conserved in the *N*-terminal sEH domain suggests that it probably has a second catalytic activity.

SUBCELLULAR LOCALISATION

As indicated by its former name cytosolic EH, sEH has first been identified in the cell cytosol (Gill *et al*. 1974). Later, a similar enzyme was found in the matrix of peroxisomes (Waechter *et al*. 1983), organelles that are separated from the cytosol by a single membrane and harbour a substantial number of different metabolic pathways, such as formation and degradation of long-chain and branched fatty acids or the degradation of urate (Lazarow and Fujiki 1985). Several of these pathways lead to the stoichiometric formation of hydrogen peroxide as a by-product, hence the name peroxisome. The presence of sEH in peroxisomes may protect the cell from secondary oxidation products generated by hydrogen peroxide and by lipid peroxidation initiated by it, but the true function is as yet unclear. Comparison of the biochemical characteristics of cytosolic and peroxisomal sEH did not reveal any significant difference between the two (Meijer and DePierre 1988; Chang and Gill 1991). Sequence analysis of the *C*-terminal of sEH appeared to explain the situation in that an imperfect carboxy terminal peroxisome targeting signal (PTS I) was identified in the rat sEH sequence (Arand *et al*. 1991), and it was concluded that the lack of perfection resulted in a reduced translocation efficacy into the peroxisomal matrix, thus leading to an unusual bi-compartmental localisation of the same enzyme. This interpretation has recently been challenged by the observation that the native sEH does not translocate into peroxisomes after recombinant expression in mammalian cells (Mullen *et al*. 1999) while a mutant with an $Ile_{554}Leu$ substitution, that restores the perfect PTS I, is exclusively localised in peroxisomes under otherwise identical conditions.

TISSUE DISTRIBUTION

The major location of sEH in most species is the liver, followed by kidney, heart, brain, lung, testes, spleen and lymphocytes (Gill and Hammock 1980; Seidegard *et al*. 1984; Schladt *et al*. 1986). At least in the organs with higher expression levels, this seems to correlate with the expression of peroxisome proliferator-activated receptor α (PPARα) (Issemann and Green 1990), the transcription factor important for the regulation of sEH expression (see below).

SPECIES DIFFERENCES

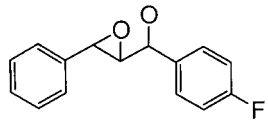
Species differences in sEH are much more pronounced than they are for mEH (Hammock *et al*. 1997). A 100-fold difference exists between the expression level of sEH in rat and mouse liver. Rat is particularly low in sEH. The sEH expression in human liver is intermediate, about 10-fold below the mouse expression level and 10-fold above the rat expression level. This suggests a significant difference between the above species with respect to the adverse effects of *trans*-substituted epoxides.

INDUCIBILITY BY FOREIGN COMPOUNDS

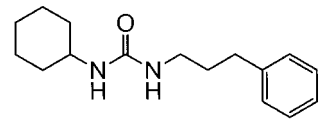
In contrast to mEH, sEH is not inducible by administration of classical inducers of xenobiotic metabolising enzymes. The only group of compounds known so far that enhances sEH expression are the peroxisome proliferators (Waechter *et al*. 1984). It was shown that in rodents, sEH expression is co-ordinately regulated with that of the enzymes involved in peroxisomal β-oxidation of fatty acids (Schladt *et al*. 1987), which are transcriptionally regulated by the PPARα (Issemann and Green 1990). A functionally active PPARα-responsive element that mediates this effect has, indeed, been identified in the rat sEH gene (Hinz W, Oesch F, and Arand M, unpublished observations).

INHIBITORS

The first established inhibitors of sEH were chalkone oxide derivatives (Mullin and Hammock 1982), the 4-fluorochalkone oxide possibly being the most important representative (Figure 12.11). Like the epoxide-derived inhibitors for mEH, these compounds are essentially low k_2 substrates for sEH (Morisseau *et al*. 1998). Very recently, alkyl urea derivatives have evolved as a novel, especially potent group of non-substrate competitive inhibitors of sEH (Morisseau *et al*. 1999b). X-ray analysis of the enzyme-inhibitor complex indicates a molecular interaction that resembles the one speculated about above for the mEH valpromide interaction (Argiriadi *et al*. 2000). The urea carbonyl seems to hydrogen bond to the active site tyrosines, while a nitrogen-bound proton hydrogen bonds to the catalytic nucleophile. These class of inhibitors possess a surprisingly low K_i—in view of the fact that they do not covalently bind to the enzyme—that is, in the nanomolar range. Thus, these compounds represent promising candidates for a possible therapeutic interaction with sEH, e.g. in prevention of multiple organ failure (see above).



4-Fluorochalcone oxide



N-Cyclohexyl-*N*'-(3-phenylpropyl)urea

Figure 12.11 Prototypes of sEH inhibitors.

GENETIC POLYMORPHISMS

Very recently, two polymorphisms affecting the protein sequence have been described for sEH (Sandberg *et al*. 2000). Of these, an $Arg_{287}Gln$ exchange that results in a surface modification of the α/β hydrolase fold domain at the dimerisation interphase seemed to have little effect on enzymic activity and protein stability, while an insertion of an additional arginine in position 402/403 seemed to decrease both, specific enzymic activity as well as protein stability. The latter change that affects a loop structure in the lid domain was proposed by the authors to slightly influence the geometry of the active centre of the enzyme. The exact prevalence of this polymorphism as well as its possible consequences for human health remain to be established.

References

Alves C, von Dippe P, Amoui M and Levy D (1993) Bile acid transport into hepatocyte smooth endoplasmic reticulum vesicles is mediated by microsomal epoxide hydrolase, a membrane protein exhibiting two distinct topological orientations. *Journal of Biological Chemistry*, **268**, 20148–20155.

Arand M, Knehr M, Thomas H, Zeller HD and Oesch F (1991) An impaired peroxisomal targeting sequence leading to an unusual bicompartmental distribution of cytosolic epoxide hydrolase. *FEBS Letters*, **294** 19–22.

Arand M, Grant DF, Beetham JK, Friedberg T, Oesch F and Hammock BD (1994) Sequence similarity of mammalian epoxide hydrolases to the bacterial haloalkane dehalogenase and other related proteins—Implication for the potential catalytic mechanism of enzymic epoxide hydrolysis. *FEBS Letters*, **338**, 251–256.

Arand M, Wagner H and Oesch F (1996) Asp^{333}, Asp^{495}, and His^{523} form the catalytic triad of rat soluble epoxide hydrolase. *Journal of Biological Chemistry*, **271**, 4223–4229.

Arand M, Hemmer H, Dürk H, Baratti J, Archelas A, Furstoss R and Oesch F (1999a) Cloning and molecular characterisation of a soluble epoxide hydrolase from Aspergillus niger that is related to mammalian microsomal epoxide hydrolase. *Biochemical Journal*, **344**, 273–280.

Arand M, Müller F, Mecky A, Hinz W, Urban P, Pompon D, Kellner R and Oesch F (1999b) Catalytic triad of microsomal epoxide hydrolase: replacement of Glu404 with Asp leads to a strongly increased turnover rate. *Biochemical Journal*, **337**, 37–43.

Argiriadi MA, Morisseau C, Hammock BD and Christianson DW (1999) Detoxification of environmental mutagens and carcinogens: structure, mechanism, and evolution of liver epoxide hydrolase. *Proceedings of the National Academy of Sciences USA*, **96**, 10637–10642.

Argiriadi MA, Morisseau C, Goodrow MH, Dowdy DL, Hammock BD and Christianson DW (2000) Binding of alkylurea inhibitors to epoxide hydrolase implicates active site tyrosines in substrate activation. *Journal of Biological Chemistry*, **275**, 15265–15270.

Armstrong RN (1987) Enzyme-catalyzed detoxication reactions: Mechanisms and stereochemistry. *CRC Critical Reviews in Biochemistry*, **22**, 39–88.

Armstrong RN (1999) Kinetic and chemical mechanism of epoxide hydrolase. *Drug Metabolism Reviews*, **3**, 71–86.

Astrom A and DePierre JW (1981) Characterisation of the induction of drug-metabolising enzymes by 2-acetylaminofluorene. *Biochimica et Biophysica Acta*, **673**, 225–233.

Backman JT, Siegle I, Zanger UM and Fritz P (1999) Immunohistochemical detection of microsomal epoxide hydrolase in human synovial tissue. *Histochemical Journal*, **31**, 645–649.

Beetham JK, Tian TG and Hammock BD (1993) cDNA cloning and expression of a soluble epoxide hydrolase from human liver. *Archives of Biochemistry and Biophysics*, **305**, 197–201.

Beetham JK, Grant D, Arand M, Garbarino J, Kiyosue T, Pinot F, Oesch F, Belknap WR, Shinozaki K and Hammock BD (1995) Gene evolution of epoxide hydrolases and recommended nomenclature. *DNA and Cell Biology*, **14**, 61–71.

Benhamou S, Reinikainen M, Bouchardy C, Dayer P and Hirvonen A (1998) Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Research*, **58**, 5291–5293.

Bentley P, Oesch F and Glatt HR (1977) Dual role of epoxide hydratase in both activation and inactivation. *Archives of Toxicology*, **39**, 65–75.

Bentley P, Waechter F, Oesch F and Staubli W (1979) Immunochemical localisation of epoxide hydratase in rat liver: effects of 2-acetylaminofluorene. *Biochemical Biophysical Research Communications*, **91**, 1101–1108.

Bogdanffy MS (1990) Biotransformation enzymes in the rodent nasal mucosa: the value of a histochemical approach. *Environmental Health Perspectives*, **85**, 177–186.

Breuer H and Knuppen R (1961) The formation of 16α,17α-epoxy-oestratriene-3-ol by rat liver tissue. *Biochimica et Biophysica Acta*, **49**, 620–621.

Chang C and Gill SS (1991) Purification and characterisation of an epoxide hydrolase from the peroxisomal fraction of mouse liver. *Archives of Biochemistry and Biophysics*, **285**, 276–284.

Craft JA, Baird S, Lamont M and Burchell B (1990) Membrane topology of epoxide hydrolase. *Biochimica et Biophysica Acta*, **1046**, 32–39.

Disse B, Siekmann L and Breuer H (1980) Biosynthesis of 16α,17α-epoxy-4-androstene-3-one in rat liver microsomes. *Acta Endocrinologica*, **95**, 58–66.

DuBois GC, Appella E, Levin W, Lu AY and Jerina DM (1978) Hepatic microsomal epoxide hydrase. Involvement of a histidine at the active site suggests a nucleophilic mechanism. *Journal of Biological Chemistry*, **253**, 2932–2939.

Eichelbaum M, Kothe KW, Hoffman F and von Unruh GE (1979) Kinetics and metabolism of carbamazepine during combined antiepileptic drug therapy. *Clinical Pharmacology and Therapeutics*, **26**, 366–371.

Etter H-U, Richter C, Ohta Y, Winterhalter KH, Sasabe H and Kawato S (1991) Rotation and interaction with epoxide hydrase of cytochrome P-450 in proteoliposomes. *Journal of Biological Chemistry*, **266**, 18600–18605.

Farin FM and Omiecinski CJ (1993) Regiospecific expression of cytochrome P-450s and microsomal epoxide hydrolase in human brain tissue. *Journal of Toxicology and Environmental Health*, **40**, 317–335.

Franken SM, Rozeboom HJ, Kalk KH and Dijkstra BW (1991) Crystal structure of haloalkane dehalogenase: an enzyme to detoxify halogenated alkanes. *EMBO Journal*, **10**, 1297–1302.

Friedberg T, Holler R, Löllmann B, Arand M and Oesch F (1996) The catalytic activity of the endoplasmic reticulum-resident protein microsomal epoxide hydrolase towards carcinogens is retained on inversion of its membrane topology. *Biochemical Journal*, **319**, 131–136.

Friedberg T, Löllmann B, Becker R, Holler R and Oesch F (1994) The microsomal epoxide hydrolase has a single membrane signal anchor sequence which is dispensable for the catalytic activity of this protein. *Biochemical Journal*, **303**, 967–972.

Gaedigk A, Leeder JS and Grant DM (1997) Tissue-specific expression and alternative splicing of human microsomal epoxide hydrolase. *DNA and Cell Biology*, **16**, 1257–1266.

Gill SS and Hammock BD (1980) Distribution and properties of a mammalian soluble epoxide hydrase. *Biochemical Pharmacology*, **29**, 389–395.

Gill SS, Hammock BD and Casida JE (1974) Mammalian metabolism and environmental degradation of the juvenoid, 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene. *Journal of Agricultural and Food Chemistry*, **22**, 386–395.

Glatt H, Jung R and Oesch F (1983) Bacterial mutagenicity investigation of epoxides: drugs, drug metabolites, steroids and pesticides. *Mutation Research*, **111**, 99–118.

Gonzalez FJ and Kasper CB (1981) Cloning of epoxide hydratase complementary DNA. *Journal of Biological Chemistry*, **256**, 4697–4700.

Grant DF, Storms DH and Hammock BD (1993) Molecular cloning and expression of murine liver soluble epoxide hydrolase. *Journal of Biological Chemistry*, **268**, 17628–17633.

Guengerich FP and Davidson NK (1982) Interaction of epoxide hydrolase with itself and other microsomal proteins. *Archives of Biochemistry and Biophysics*, **215**, 462–477.

Guenthner TM and Karnezis TA (1986) Immunochemical characterisation of human lung epoxide hydrolases. *Journal of Biochemical Toxicology*, **1**, 67–81.

Guenthner TM, Hammock BD, Vogel U and Oesch F (1981) Cytosolic and microsomal epoxide hydrolases are immunologically distinguishable from each other in the rat and mouse. *Journal of Biological Chemistry*, **256**, 3163–3166.

Guenthner TM, Cai D and Wallin R (1998) Co-purification of microsomal epoxide hydrolase with the warfarin-sensitive vitamin K1 oxide reductase of the vitamin K cycle. *Biochemical Pharmacology*, **55**, 169–175.

Haeggstrom JZ, Wetterholm A, Vallee BL and Samuelsson B (1990) Leukotriene A4 hydrolase: an epoxide hydrolase with peptidase activity. *Biochemical Biophysical Research Communications*, **173**, 431–437.

Hammock BD, Storms DH and Grant DF, 1997, Epoxide hydrolases. In *Biotransformation*, Guengerich FP (ed.), Elsevier Sciences, New York, pp. 283–305.

Hassett C, Aicher L, Sidhu JS and Omiecinski CJ (1994) Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Human Molecular Genetics*, **3**, 421–428.

Hassett C, Lin J, Carty CL, Laurenzana EM and Omiecinski CJ (1997) Human hepatic microsomal epoxide hydrolase: comparative analysis of polymorphic expression. *Archives of Biochemistry and Biophysics*, **337**, 275–283.

Hattori N, Fujiwara H, Maeda M, Fujii S and Ueda M (2000) Epoxide hydrolase affects oestrogen production in the human ovary. *Endocrinology*, **141**, 3353–3365.

Hayes JD and Pulford DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology*, **30**, 445–600.

Heinemann FS and Ozols J (1984) The covalent structure of microsomal epoxide hydrolase. II. The complete amino acid sequence. *Journal of Biological Chemistry*, **259**, 797–804.

Herrero ME, Arand M, Hengstler JG and Oesch F (1997) Recombinant expression of human microsomal epoxide hydrolase protects V79 Chinese hamster cells from styrene oxide—but not from ethylene oxide-induced DNA strand breaks. *Environmental Molecular Mutagenesis*, **30**, 429–439.

Holder G, Yagi H, Dansette P, Jerina DM, Levin W, Lu AY and Conney AH (1974) Effects of inducers and epoxide hydrase on the metabolism of benzo(a)pyrene by liver microsomes and a reconstituted system: analysis by high pressure liquid chromatography. *Proceedings of the National Academy of Sciences, USA* **71**, 4356–4360.

Holler R, Arand M, Mecky A, Oesch F and Friedberg T (1997) The membrane anchor of microsomal epoxide hydrolase from human, rat, and rabbit displays an unexpected membrane topology. *Biochemical Biophysical Research Communications*, **236**, 754–759.

Honscha W, Oesch F and Friedberg T (1991) Tissue-specific expression and differential inducibility of several microsomal epoxide hydrolase mRNAs which are formed by alternative splicing. *Archives of Biochemistry and Biophysics*, **287**, 380–385.

Honscha W, Platte HD, Oesch F and Friedberg T (1995) Relationship between the microsomal epoxide hydrolase and the hepatocellular transport of bile acids and xenobiotics. *Biochemical Journal*, **311**, 975–979.

Issemann I and Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*, **347**, 645–650.

Janssen DB, Fries F, van der Ploeg J, Kazemier B, Terpstra P and Witholt B (1989) Cloning of 1,2-dichloroethane degradation genes of *Xanthobacter autotrophicus* GJ10 and expression and sequencing of the *dhlA* gene. *Journal of Bacteriology*, **171**, 6791–6799.

Jenkins Sumner S and Fennell TR (1994) Review on the metabolic fate of styrene. *Critical Reviews in Toxicology*, **24**, S11–S33.

Jernstrom B, Seidel A, Funk M, Oesch F and Mannervik B (1992) Glutathione conjugation of trans-3,4-dihydroxy 1,2-epoxy 1,2,3,4-tetrahydrobenzo[c]phenanthrene isomers by human glutathione transferases. *Carcinogenesis*, **13**, 1549–1555.

Kessler W, Jiang X and Filser JG (1992) Pharmakokinetik von Styrol-7,8-Oxid bei Maus und Ratte. In *Arbeitsmedizinische Aspekte der Arbeits(-zeit)organisation—Skeletterkrankungen und Beruf—Arbeitsmedizinisches Kolloquium der gewerblichen Berufsgenossenschaften*, Kreutz R and Piekarski C (eds), Gentner Verlag, Stuttgart, pp. 622–626.

Kessler R, Hamou MF, Albertoni M, de Tribolet N, Arand M and Van Meir EG (2000)

Identification of the putative brain tumor antigen BF7/GE2 as the (de)toxifying enzyme microsomal epoxide hydrolase. *Cancer Research*, **60**, 1403–1409.

Kim SG, Cho JY and Jung KH (1995) Differential expression of rat microsomal epoxide hydrolase gene by imidazole and triazole antimycotic agents. *Drug Metabolism and Disposition*, **23**, 460–464.

Kiyosue T, Beetham JK, Pinot F, Hammock BD, Yamaguchi-Shinozaki K and Shinozaki K (1994) Characterisation of an *Arabidopsis* cDNA for a soluble epoxide hydrolase gene that is inducible by auxin and water stress. *Plant Journal*, **6**, 259–269.

Knehr M, Thomas H, Arand M, Gebel T, Zeller HD and Oesch F (1993) Isolation and characterisation of a cDNA encoding rat liver cytosolic epoxide hydrolase and its functional expression in Escherichia-Coli. *Journal of Biological Chemistry*, **268**, 17623–17627.

Koonin EV and Tatusov RL (1994) Computer analysis of bacterial haloacid dehalogenases defines a large superfamily of hydrolases with diverse specificity—application of an iterative approach to database search. *Journal of Molecular Biology*, **244**, 125–132.

Krämer A, Frank H, Setiabudi F, Oesch F and Glatt H (1991) Influence of the level of cytosolic epoxide hydrolase on the induction of sister chromatid exchanges by trans-beta-ethylstyrene 7,8-oxide in human lymphocytes. *Biochemical Pharmacology*, **42**, 2147–2152.

Lacourciere GM and Armstrong RN (1993) The catalytic mechanism of microsomal epoxide hydrolase involves an ester intermediate. *Journal of the American Chemical Society*, **115**, 10466–10467.

Lacourciere GM and Armstrong RN (1994) Microsomal and soluble epoxide hydrolases are members of the same family of C–X bond hydrolase enzymes. *Chemical Research in Toxicology*, **7**, 121–124.

Lancaster JM, Brownlee HA, Bell DA, Futreal PA, Marks JR, Berchuck A, Wiseman RW and Taylor JA (1996) Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Molecular Carcinogenesis*, **17**, 160–162.

Laughlin LT, Tzeng H-F, Lin S and Armstrong RN (1998) Mechanism of microsomal epoxide hydrolase. Semifunctional site-specific mutants affecting the alkylation half-reaction. *Biochemistry*, **37**, 2897–2904.

Lazarow PB and Fujiki Y (1985) Biogenesis of peroxisomes. *Annual Review of Cell Biology*, **1**, 489–530.

Levin W, Michaud DP, Thomas PE and Jerina DM (1983) Distinct rat hepatic microsomal epoxide hydrolases catalyze the hydration of cholesterol 5,6 alpha-oxide and certain xenobiotic alkene and arene oxides. *Archives of Biochemistry and Biophysics*, **220**, 485–494.

Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, Lancet D, Louisot P, Magdalou J, Chowdhury JR, Ritter JK, Schachter H, Tephly TR, Tipton KF and Nebert DW (1997) The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics*, **7**, 255–269.

McGlynn KA, Rosvold EA, Lustbader ED, Hu Y, Clapper ML, Zhou T, Wild CP, Xia XL, Baffoe-Bonnie A, Ofori-Adjei D, Chen GC, London WT, Shen FM and Buetow K (1995) Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymic detoxification of aflatoxin B1. *Proceedings of the National Academy of Sciences USA*, **92**, 2384–2387.

Meijer J and DePierre JW (1988) Immunoaffinity purification and comparison of epoxide hydrolases from liver cytosol and peroxisomes of untreated and clofibrate-treated mice. *Archives of Toxicology*, **Suppl. 12**, 283–287.

Meijer JW, Binnie CD, Debets RM, van Parys JA and de Beer-Pawlikowski NK (1984) Possible hazard of valpromide-carbamazepine combination therapy in epilepsy. *Lancet*, **8380**, 802.

Mertes I, Fleischmann R and Oesch F (1985) Interindividual variations in the activities of cytosolic and microsomal epoxide hydrolase in human liver. *Carcinogenesis*, **6**, 219–223.

Mesange F, Sebbar M, Kedjouar B, Capdevielle J, Guillemot JC, Ferrara P, Bayard F, Delarue F, Faye JC and Poirot M (1998) Microsomal epoxide hydrolase of rat liver is a subunit of the anti-oestrogen-binding site. *Biochemical Journal*, **334**, 107–112.

Miller EC and Miller JA (1981) Mechanisms of chemical carcinogenesis. *Cancer*, **47**, 1055–1064.

Miyata M, Kudo G, Lee YH, Yang TJ, Gelboin HV, Fernandez-Salguero P, Kimura S and Gonzalez FJ (1999) Targeted disruption of the microsomal epoxide hydrolase gene. Microsomal epoxide hydrolase is required for the carcinogenic activity of 7,12-dimethylbenz[a]anthracene. *Journal of Biological Chemistry*, **274**, 23963–23968.

Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC and Hammock BD (1997) Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nature Medicine*, **3**, 562–566.

Morisseau C, Du G, Newman JW and Hammock BD (1998) Mechanism of mammalian soluble epoxide hydrolase inhibition by chalcone oxide derivatives. *Archives of Biochemistry and Biophysics*, **356**, 214–228.

Morisseau C, Archelas A, Guitton C, Faucher D, Furstoss R and Baratti JC (1999a) Purification and characterisation of a highly enantioselective epoxide hydrolase from Aspergillus niger. *European Journal of Biochemistry*, **263**, 386–395.

Morisseau C, Goodrow MH, Dowdy D, Zheng J, Greene JF, Sanborn JR and Hammock BD (1999b) Potent urea and carbamate inhibitors of soluble epoxide hydrolases. *Proceedings of the National Academy of Sciences, USA*, **96**, 8849–8854.

Mullen RT, Trelease RN, Duerk H, Arand M, Hammock BD, Oesch F and Grant DF (1999) Differential subcellular localisation of endogenous and transfected soluble epoxide hydrolase in mammalian cells: evidence for isozyme variants. *FEBS Letters*, **445**, 301–305.

Mullin CA and Hammock BD (1982) Chalcone oxides—Potent selective inhibitors of cytosolic epoxide hydrolase. *Archives of Biochemistry and Biophysics*, **216**, 423–439.

Nardini M, Ridder IS, Rozeboom HJ, Kalk KH, Rink R, Janssen DB and Dijkstra BW (1999) The x-ray structure of epoxide hydrolase from Agrobacterium radiobacter AD1. An enzyme to detoxify harmful epoxides. *Journal of Biological Chemistry*, **274**, 14579–14586.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, **6**, 1–42.

Nourooz-Zadeh J, Uematsu T, Borhan B, Kurth MJ and Hammock BD (1992) Characterisation of the epoxide hydrolase-catalyzed hydration products from 9,10:12,13-diepoxy stearic esters. *Archives of Biochemistry and Biophysics*, **294**, 675–685.

Oesch F (1973) Mammalian epoxide hydrases: Inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. *Xenobiotica*, **3**, 305–340.

Oesch F and Arand M, (1994), Induction of drug-metabolising enzymes by short/intermediate-term exposure to peroxisome proliferators: a synopsis. In *Peroxisome Proliferators: Unique Inducers of Drug-Metabolizing Enzymes*, Moody DE (ed.), CRC Press, Boca Raton, FL, pp. 161–174.

Oesch F and Bentley P (1976) Antibodies against homogeneous epoxide hydratase provide evidence for a single enzyme hydrating styrene oxide and benz(a)pyrene 4,5-oxide. *Nature*, **259**, 53–55.

Oesch F and Daly J (1972) Conversion of naphthalene to trans -naphthalene dihydrodiol: Evidence for the presence of a coupled aryl monooxygenase-epoxide hydrase system in hepatic microsomes. *Biochemical Biophysical Research Communications*, **46**, 1713–1720.

Oesch F and Golan M (1980) Specificity of mouse liver cytosolic epoxide hydrolase for K-region epoxides derived from polycyclic aromatic hydrocarbons. *Cancer Letters*, **9**, 169–175.

Oesch F, Jerina DM and Daly J (1971a) A radiometric assay for hepatic epoxide hydrase activity with 7-3H-styrene oxide. *Biochimica et Biophysica Acta*, **227**, 685–691.

Oesch F, Kaubisch N, Jerina DM and Daly J (1971b) Hepatic epoxide hydrase: Structure-activity relationships for substrates and inhibitors. *Biochemistry*, **10**, 4858-4866.

Oesch F, Glatt HR and Schmassmann HU (1977a) The apparent ubiquity of epoxide hydratase in rat organs. *Biochemical Pharmacology*, **26**, 603–607.

Oesch F, Raphael D, Schwind H and Glatt HR (1977b) Species differences in activating and inactivating enzymes related to the control of mutagenic metabolites. *Archives of Toxicology*, **39**, 97–108.

Oesch F, Timms CW, Walker CH, Guenthner TM, Sparrow A, Watabe T and Wolf CR (1984)

Existence of multiple forms of microsomal epoxide hydrolases with radically different substrate specificities. *Carcinogenesis*, **5**, 7–9.
Oesch F, Herrero ME, Hengstler JG, Lohmann M and Arand M (2000) Metabolic detoxification: implications for thresholds. *Toxicological Pathology*, **28**, 382–387.
Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, Harel M, Remington SJ, Silman I, Schrag J, Sussman JL, Verschueren KHG and Goldman A (1992) The α/β hydrolase fold. *Protein Engineering*, **5**, 197–211.
Ota K and Hammock BD (1980) Cytosolic and microsomal epoxide hydrolases: Differential properties in mammalian liver. *Science*, **207**, 1479–1481.
Ozawa T, Hayakawa M, Kosaka K, Sugiyama S, Ogawa T, Yokoo K, Aoyama H and Izawa Y (1991) Leukotoxin, 9,10-epoxy-12-octadecenoate, as a burn toxin causing adult respiratory distress syndrome. *Advances in Prostaglandin, Thromboxane and Leukotoxin Research*, **21B**, 569–572.
Pace-Asciak CR and Lee WS (1989) Purification of hepoxilin epoxide hydrolase from rat liver. *Journal of Biological Chemistry*, **264**, 9310–9313.
Papadopoulos D, Jornvall H, Rydstrom J and Depierre JW (1994) Purification and initial characterisation of microsomal epoxide hydrolase from the human adrenal gland. *Biochimica et Biophysica Acta*, **1206**, 253–262.
Pinot F, Grant DF, Beetham JK, Parker AG, Borhan B, Landt S, Jones AD and Hammock BD (1995) Molecular and biochemical evidence for the involvement of the Asp-333- His-523 pair in the catalytic mechanism of soluble epoxide hydrolase. *Journal of Biological Chemistry*, **270**, 7968–7974.
Ponomarkov V, Cabral JR, Wahrendorf J and Galendo D (1984) A carcinogenicity study of styrene-7,8-oxide in rats. *Cancer Letters*, **24**, 95–101.
Porter TD, Beck TW and Kasper CB (1986) Complementary DNA and amino acid sequence of rat liver microsomal, xenobiotic epoxide hydrolase. *Archives of Biochemistry and Biophysics*, **248**, 121–129.
Pries F, Kingma J, Pentenga M, Vanpouderoyen G, Jeronimusstratingh CM, Bruins AP and Janssen DB (1994) Site-Directed mutagenesis and oxygen isotope incorporation studies of the nucleophilic aspartate of haloalkane dehalogenase. *Biochemistry*, **33**, 1242–1247.
Raaka S, Hassett C and Omiencinski CJ (1998) Human microsomal epoxide hydrolase: 5′-flanking region genetic polymorphisms. *Carcinogenesis*, **19**, 387–393.
Rappaport SM, Yeowell-O'Connell K, Bodell W, Yager JW and Symanski E (1996) An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. *Cancer Research*, **56**, 5410–5416.
Rink R, Fennema M, Smids M, Dehmel U and Janssen DB (1997) Primary structure and catalytic mechanism of the epoxide hydrolase from *Agrobacterium radiobacter* AD1. *Journal of Biological Chemistry*, **272**, 14650–14657.
Rink R, Kingma J, Lutje Spelberg JH and Janssen DB (2000) Tyrosine residues serve as proton donor in the catalytic mechanism of epoxide hydrolase from Agrobacterium radiobacter. *Biochemistry*, **39**, 5600–5613.
Sandberg M, Hassett C, Adman ET, Meijer J and Omiecinski CJ (2000) Identification and functional characterisation of human soluble epoxide hydrolase genetic polymorphisms. *Journal of Biological Chemistry*, **275**, 28873–28881.
Schladt L, Woerner W, Setiabudi F and Oesch F (1986) Distribution and inducibility of cytosolic epoxide hydrolase in male Sprague-Dawley rats. *Biochemical Pharmacology*, **35**, 3309–3316.
Schladt L, Hartmann R, Timms C, Strolin-Benedetti M, Dostert P, Wörner W and Oesch F (1987) Concomitant induction of cytosolic but not microsomal epoxide hydrolase with peroxisomal β-oxidation by various hypolipidemic compounds. *Biochemical Pharmacology*, **36**, 345–351.
Schmassmann HU and Oesch F (1978) *Trans*-stilbene oxide: A selective inducer of rat liver epoxide hydratase. *Molecular Pharmacology*, **14**, 834–847.
Seidegard J, DePierre JW and Pero RW (1984) Measurement and characterisation of membrane-bound and soluble epoxide hydrolase activities in resting mononuclear leukocytes from human blood. *Cancer Research*, **44**, 3654–3660.
Sheehan JE, Pitot HC and Kasper CB (1991) Transcriptional regulation and localisation of the

tissue-specific induction of epoxide hydrolase by lead acetate in rat kidney. *Journal of Biological Chemistry*, **266**, 5122–5127.
Sims P, Grover PL, Swaisland A, Pal K and Hewer A (1974) Metabolic activation of benzo(a)-pyrene proceeds by a diol-epoxide. *Nature*, **252**, 326–328.
Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR and Gonzalez FJ (2000) Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *Journal of Biological Chemistry*, in press.
Stapleton A, Beetham JK, Pinot F, Garbarino JE, Rockhold DR, Friedman M, Hammock BD and Belknap WR (1994) Cloning and expression of soluble epoxide hydrolase from potatoe. *Plant Journal*, **6**, 251–258.
Steinberg P, Lafranconi WM, Wolf CR, Waxman DJ, Oesch F and Friedberg T (1987) Xenobiotic metabolising enzymes are not restricted to parenchymal cells in rat liver. *Molecular Pharmacology*, **32**, 463–470.
Thomas H, Timms CW and Oesch F (1990), Epoxide hydrolases: Molecular properties, induction, polymorphisms and function. In *Frontiers in Biotransformation*, Ruckpaul K and Rein H (eds), Akademie-Verlag, Berlin, pp. 278–337.
Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.
Tzeng H-F, Laughlin LT, Lin S and Armstrong RN (1996) The catalytic mechanism of microsomal epoxide hydrolase involves reversible formation and rate-limiting hydrolysis of the alkyl-enzyme intermediate. *Journal of the American Chemical Society*, **118**, 9436–9437.
Tzeng H-F, Laughlin LT and Armstrong RN (1998) Semifunctional site-specific mutants affecting the hydrolytic half-reaction of microsomal epoxide hydrolase. *Biochemistry*, **37**, 2905–2911.
Verschueren KHG, Seljée F, Rozeboom HJ, Kalk KH and Dijkstra BW (1993) Crystallographic analysis of the catalytic mechanism of haloalkane dehalogenase. *Nature*, **363**, 693–698.
Vogel-Bindel U, Bentley P and Oesch F (1982) Endogenous role of microsomal epoxide hydrolase: ontogenesis, induction, inhibition, tissue distribution, immunological behaviour and purification of microsomal epoxide hydrolase with 16α,17α-epoxy-androstene3-one as substrate. *European Journal of Biochemistry*, **126**, 425–431.
von Dippe P, Amoui M, Alves C and Levy D (1993) Na(+)-dependent bile acid transport by hepatocytes is mediated by a protein similar to microsomal epoxide hydrolase. *American Journal of Physiology*, **264**, G528–534.
von Dippe P, Amoui M, Stellwagen RH and Levy D (1996) The functional expression of sodium-dependent bile acid transport in Madin-Darby canine kidney cells transfected with the cDNA for microsomal epoxide hydrolase. *Journal of Biological Chemistry*, **27**, 18176–18180.
Waechter F, Bentley P, Germann M, Oesch F and Stäubli W (1982) Immuno-electron-microscopic studies on the subcellular distribution of rat liver epoxide hydrolase and the effect of phenobarbitone and 2-acetamidofluorene treatment. *Biochemical Journal*, **202**, 677–686.
Waechter F, Bentley P, Bieri F, Stäubli W, Völkl A and Fahimi HD (1983) Epoxide hydrolase activity in isolated peroxisomes of mouse liver. *FEBS Letters*, **158**, 225–228.
Waechter F, Bieri F, Stäubli W and Bentley P (1984) Induction of cytosolic and microsomal epoxide hydrolases by the hypolipidaemic compound nafenopin in the mouse liver. *Biochemical Pharmacology*, **33**, 31–34.
Williams PA, Cosme J, Sridhar V, Johnson EF and McRee DE (2000) Mammalian microsomal cytochrome P450 monooxygenase: structural adaptations for membrane binding and functional diversity. *Molecular Cell*, **5**, 121–131.
Wilson R, Ainscough R, Anderson K, Baynes C, Berks M, Bonfield J, Burton J, Connell M, Copsey T, Cooper J, Coulson A, Craxton M, Dear S, Du Z, Durbin R, Favello A, Fulton L, Gardner A, Green P, Hawkins T, Hillier L, Jier M, Johnston L, Jones M, Kershaw J, Kirsten J, Laister N, Latreille P, Lightning J, Lloyd C, McMurray A, Mortimore B, O'Callaghan M, Parsons J, Percy C, Rifken L, Roopra A, Saunders D, Shownkeen R, Smaldon N, Smith A, Sonnhammer E, Staden R, Sulston J, Thierry-Mieg J, Thomas K, Vaudin M, Vaughan K, Waterston R, Watson A, Weinstock L, Wilkinson-Sproat J and Wohldman P (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans. *Nature*, **368**, 32–38.
Wojtasek H and Prestwich GD (1996) An insect juvenile hormone-specific epoxide hydrolase is

related to vertebrate microsomal epoxide hydrolase. *Biochemical Biophysical Research Communications*, **220**, 323–329.

Wolf CR, Buchmann A, Friedberg T, Moll E, Kuhlmann WD, Kunz HW and Oesch F (1984) Dynamics of the localisation of drug metabolising enzymes in tissues and cells. *Biochemical Society Transactions*, **12**, 60–62.

Yamada T, Morisseau C, Maxwell JE, Argiriadi MA, Christianson DW and Hammock BD (2000) Biochemical evidence for the involvement of tyrosine in epoxide activation during the catalytic cycle of epoxide hydrolase. *Journal of Biological Chemistry*, **275**, 23082–23088.

Zeldin DC, Kobayashi J, Falck JR, Winder BS, Hammock BD, Snapper JR and Capdevila JH (1993) Regiofacial and enantiofacial selectivity of epoxyeicosatrienoic acid hydration by cytosolic epoxide hydrolase. *Journal of Biological Chemistry*, **268**, 6402–6407.

Zhu Q, von Dippe P, Xing W and Levy D (1999) Membrane topology and cell surface targeting of microsomal epoxide hydrolase. Evidence for multiple topological orientations. *Journal of Biological Chemistry*, **274**, 27898–27904.

Zou J, Hallberg BM, Bergfors T, Oesch F, Arand M, Mowbray SL and Jones TA (2000) Structure of Aspergillus niger epoxide hydrolase at 1.8 A resolution: implications for the structure and function of the mammalian microsomal class of epoxide hydrolases. *Structure with Folding & Design*, **8**, 111–122.