Biochemical Pharmacology, Vol. 41, No. 11, pp. 1625–1637, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

EFFECTS OF ENVIRONMENTALLY ENCOUNTERED EPOXIDES ON MOUSE LIVER EPOXIDE-METABOLIZING ENZYMES

DAVID E. MOODY,*†‡ KAREN A. MONTGOMERY,*§ MOHAMED B. A. ASHOUR*‖ and BRUCE D. HAMMOCK*

*Departments of Entomology and Environmental Toxicology, University of California, Davis, CA 95616; and †Center for Human Toxicology, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, U.S.A.

(*Received* 11 *September* 1990; *accepted* 13 *December* 1990)

Abstract—Male mice were treated (i.p.) for 3 days with 15 different environmentally encountered epoxides, and the effects of these compounds on liver microsomal and cytosolic epoxide hydrolase (mEH and cEH), glutathione *S*-transferase (mGST and cGST) and carboxylesterase (mCE) activities were determined. The epoxides included the pesticides: heptachlor epoxide, dieldrin, tridiphane, and juvenoid R-20458; the natural products: disparlure, limonin, nomilin, and epoxymethyloleate; the endogenous steroids: lanosterol epoxide, cholesterol-α-epoxide, and progesterone epoxide; and the industrial or synthetic epoxides: epichlorohydrin, araldite, *trans*-stilbene oxide, and 4′-phenylchalcone oxide. The pesticide epoxides were the most effective inducers of liver weight, microsomal protein, and the enzyme activities measured, with mEH and cEH activities towards *cis*-stilbene oxide (mEHcso and cEHcso), cGST activities towards four of five substrates, and mCE towards clofibrate (mCEclof) and *p*-nitrophenylacetate (mCEpna) increased following treatment with most of the pesticides. The synthetic epoxides increased some of the same activities, while the natural products, except for increases in cGST activities, and endogenous steroid epoxides were generally not inductive. cEH activity towards *trans*-stilbene oxide (cEHtso) was increased only following treatment with the peroxisome proliferator, tridiphane, but decreased following treatment with several of the epoxides, while microsomal cholesterol epoxide hydrolase (mEHchol) was increased only moderately by disparlure. Microsomes could effectively conjugate glutathione to chlorodinitrobenzene (mGSTcdnb) and *cis*-stilbene oxide (mGSTcso). These two activities were differentially induced by a few of the epoxides, suggesting that they may be selective substrates for different isozymes of mGST. Correlation coefficients were determined for the relative response of liver weight, subfraction protein, and enzyme activities. A relatively high correlation was found between the response of liver weight and cytosolic hydrolysis of *trans*-stilbene oxide ($r = 0.73$) and *cis*-stilbene oxide ($r = 0.62$), and cytosolic glutathione conjugation of dichloronitrobenzene ($r = 0.66$) and *trans*-stilbene oxide ($r = 0.75$). In addition, relatively high correlations were found between the different cGST activities, in particular for dichloronitrobenzene with *trans*-stilbene oxide ($r = 0.89$). These studies show that there exists a wide variation in the response of xenobiotic-metabolizing enzymes to environmentally encountered epoxides and that a fairly strong correlation exists between the increases in liver size and increases in certain cytosolic enzyme activities; they also suggest further studies concerning the possibility of an additional isozyme of mGST.

Chemicals containing the epoxide functionality are distributed throughout mankind's environment [1], but little is known concerning how exposure to them alters their own metabolism. Three-membered cyclic ethers, epoxides are also referred to as oxiranes, or oxides of olefinic and aromatic compounds. Cleavage of the ether bond in the presence of electrophiles is readily facilitated for a number of epoxides, and often results in adduct formation. As a result, epoxides have been implicated as the proximate toxin or mutagen for large number of xenobiotics [2–4]. For many of these, the epoxide is introduced during the oxidative metabolism of the xenobiotic. Epoxides, many of which are stable enough to reach biologically susceptible targets, also abound within the environment.

The disposition of epoxides within biological systems is partially a function of their metabolism. Two main enzyme systems are responsible for the metabolism of epoxides. These are the epoxide hydrolases (EH; EC 3.3.2.3) and glutathione *S*-transferases (GST; EC 2.5.1.18). The GSTs are a family of related isozymes. Three distinct isozymes have been found in mouse liver cytosol [5], with upwards to seven or more distinct isozymes described in other mammalian species [6, 7]. GSTs are primarily localized in the cytosol, but activity for at least one distinct isozyme has been found in liver and kidney microsomes [8, 9].

In mammals, evidence has been presented for the existence of at least four distinct EHs. Two of these are apparently not involved in xenobiotic metabolism and include a soluble EH specific for leukotriene A_4, and a microsomal EH specific for Δ-5 steroid

‡ Address for correspondence: David E. Moody, Ph.D., Center for Human Toxicology, University of Utah, 417 Wakara Way, Rm. 290, Salt Lake City, UT 84108.

§ Current address: Graduate School of Public Health, San Diego State University, San Diego, CA 92182.

‖ Permanent address: Department of Plant Protection, College of Agriculture, University of Zagazig, Zagazig, Egypt.

epoxides. The other two EHs have broad substrate specificity for a number of endogenous and xenobiotic epoxides. Based upon their primary subcellular localization, these two xenobiotic-metabolizing EHs are referred to as the microsomal and cytosolic EH [10–12]. The microsomal and cytosolic EH have been shown to be immunochemically distinct, and selective substrates and inhibitors for each have been identified. In regard to the former, *cis*-stilbene oxide (CSO) and benzo[*a*]pyrene-4,5-oxide are selective for the microsomal EH, while *trans*-stilbene oxide (TSO) is selective for the cytosolic CH* [13, 14]. In a species-dependent manner, the subcellular localizations of these two EHs are not restricted. EH with immunochemical and substrate specificity similar to the microsomal EH has been described in nuclear envelopes and serum, and EH with immunochemical and substrate specificity similar to the cytosolic EH has been described in microsomes, peroxisomes, nuclear membranes, and mitochondria. Based upon substrate specificity, evidence has also been presented for microsomal-like EH in liver cytosol [10, 12].

It is now well recognized that the activity and content of many of the xenobiotic-metabolizing enzymes, including the EHs and the GSTs, can be modulated by xenobiotics within the environment [6, 7, 10–12]. In many cases, this modulation can affect the metabolic disposition of the xenobiotic itself, as well as other xenobiotic and endogenous compounds, with ensuing effects on their pharmaco- and toxico-dynamic effects. While the responses of mammalian microsomal and cytosolic EHs to model epoxides have been studied [15], little is known about the effects of environmentally encountered epoxides on EHs and GSTs. We have studied the responses of microsomal and cytosolic EH and GST activities to acute 3-day treatment of male mice with fifteen different pesticide, natural product, endogenous steroid, and industrial epoxides. Microsomal carboxylesterases (CE; EC 3.1.1.1) were also chosen for study. This set of xenobiotic-metabolizing enzymes are not routinely involved in epoxide metabolism, but little is known of the capacity of these enzymes to respond to xenobiotic treatments [16, 17]. Studies which perturb enzyme activities with a number of different treatments offer an opportunity to search for correlations between the responses found. This proved useful in analysis of the divergent responses of epoxide-metabolizing enzymes to environmentally encountered epoxides.

MATERIALS AND METHODS

Epoxides. The structures of the epoxides employed in this study are shown in Fig. 1. The following epoxides were obtained from the commercial sources listed with their stated purity given in parentheses. Heptachlor epoxide (99%) (1,4,5,6,7,8,8-heptochloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan) and dieldrin (99%) (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,exo-1,4:5,8-dimethanonaphthalene) were purchased from Chem Services, Inc. (West Chester, PA). Disparlure (90%) [(±)*cis*-7,8-epoxy-2-methyloctadecane] and *trans*-stilbene oxide (99%) were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Cholesterol-α-epoxide (TLC one-spot) (cholestan-5,6α-epoxy-3β-ol) and progesterone epoxide (TLC one-spot) (4-pregnen-16α,17α-epoxy-3,20-dione) were purchased from Steraloids, Inc. (Wilton, NH). Epichlorohydrin (99%) was purchased from the Eastman Kodak Co. (Rochester, NY). Tridiphane (99%) [2-(3,5 dichlorophenyl)-2(2,2,2-trichloroethyl)oxirane] was provided by the Dow Chemical Co. (Midland, MI). Araldite MY 790, the diglycidyl ether of bis-phenol A (isopropylidenediphenol-diglycidyl ether) was provided by CIBA-GEIGY AG (Basel, Switzerland). Limonin (limonoic acid 3,19:16,17-dilactone) and nomilin [8,(3-furyl)-decahydro-2,2,4a,8a-tetramethyl-11*H*,13*H*-oxireno-[d]pyrano[4′,3′:3,3a]isobenzofuro-[5,4-f][2]benzopyran-4,6-13(2*H*,5a*H*)-trione] were provided by David Dreyer, USDA (Albany, CA), and had been extracted from grapefruit seeds with purification including column chromatography and crystallization, with identification verified by i.r. spectrometry. Lanosterol epoxide, juvenoid R-20458, epoxymethyloleate and 4′-phenylchalcone oxide were prepared in this laboratory, produced single spots after TLC separation in two different elution systems, and reacted with the epoxide selective reagent 4-(nitrobenzyl)pyridine. Their structures were verified by NMR, i.r., and mass spectrometry. Lanosterol epoxide was prepared by peracid oxidation of purified lanosterol as described [18]. The juvenoid R-20458 [19], epoxymethyloleate [20], and 4′-phenylchalcone oxide [21] were prepared as previously described.

Other chemicals. 3,4-Dichloronitrobenzene (DCNB), *p*-nitrophenylacetate (PNPA), isooctane (99%), and hexanol (98%) were purchased from the Aldrich Chemical Co. Bovine serum albumin, 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP), and reduced glutathione were purchased from the Sigma Chemical Co. (St. Louis, MO). Bio-Rad protein reagent was purchased from Bio-Rad Laboratories (Richmond, CA). ACS scintillation fluid was purchased from Amersham (Arlington Heights, IL). Clofibrate (CLOF) was provided by Ayerst Laboratories (New York, NY), malathion (MAL) by M. Mallipudi, American Cyanamid Corp. (Princeton, NJ) and [^{14}C]cholesterol-α-epoxide (CHOL) by Alex Sevanian (School of Pharmacy, University of Southern California). CSO and tritiated CSO and TSO were prepared as previously described [22].

Animals and treatments. Male Swiss–Webster mice (Bantin–Kingman, Fremont, CA) weighing 25–30 g were housed in steel cages with kiln-dried pine shavings as bedding in an environmentally controlled

* The enzyme activities described in this study are designated by the cell fraction given in lower case letters (m, microsomal; c, cytosolic), the generic abbreviation of the enzyme given in upper case letters (EH, epoxide hydrolase; GST, glutathione *S*-transferase; CE, carboxylesterase), and the substrate given in lower case letters (cso, *cis*-stilbene oxide; tso, *trans*-stilbene oxide, etc.). Thus, the microsomal epoxide hydrolase activity towards *cis*-stilbene oxide is abbreviated as mEHcso. When the substrates are mentioned alone, they are given in upper case letters (CSO, TSO, etc.).

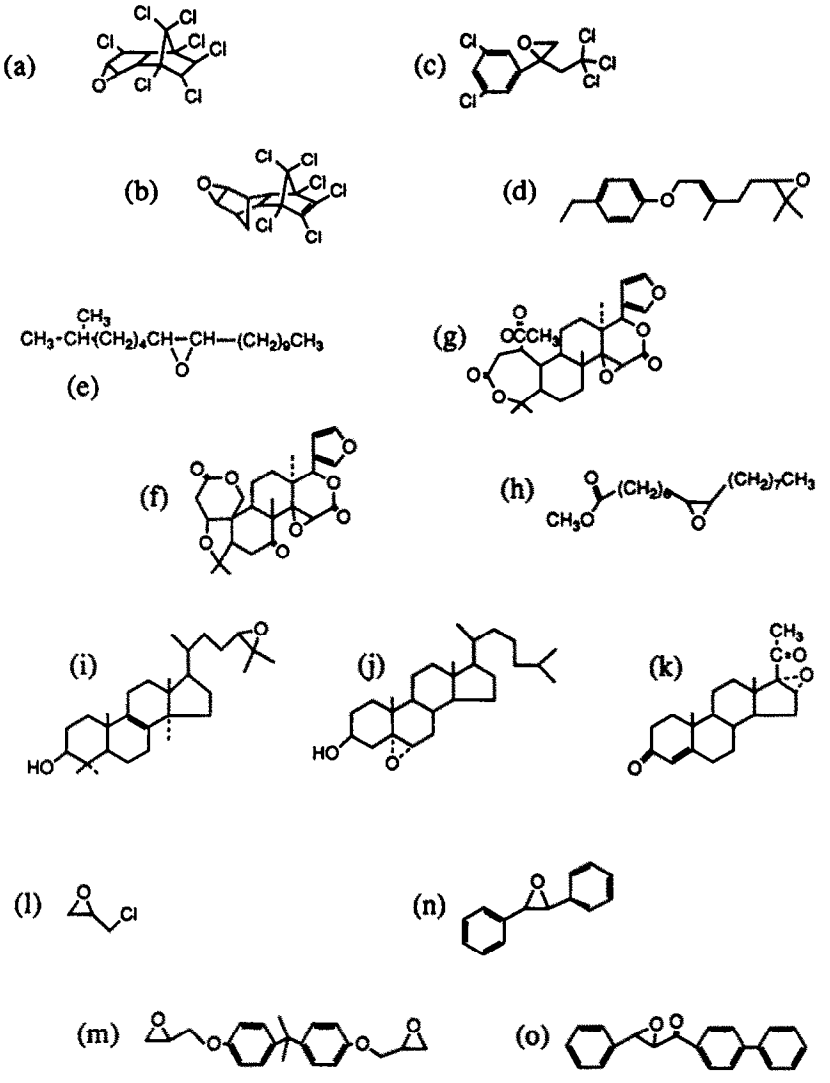

Fig. 1. Structures of the (a–d) pesticide, (e–h) natural product, (i–k) endogenous steroid, and (l–o) industrial/synthetic epoxides that were used for treatments. The individual epoxides, and their abbreviations as used in subsequent figures (given in parentheses), are as follows: (a) heptachlor epoxide (HE), (b) dieldrin (DLN), (c) tridiphane (TRD), (d) juvenoid R-20458 (R-2), (e) disparlure (DIS), (f) limonin (LIM), (g) nomilin (NOM), (h) epoxymethyloleate (EMO), (i) lanosterol epoxide (LaE), (j) cholesterol-α-epoxide (ChE), (k) progesterone epoxide (PrE), (l) epichlorohydrin (EPI), (m) araldite (ARA), (n) *trans*-stilbene oxide (TSO), and (o) 4'-phenylchalcone oxide (PCO).

room (12-hr light cycle, 22.5 to 24.0°, constant humidity). Free access was provided to food and tap water. Nine to twelve mice were treated as a group. Each group included one set of control mice and two or three sets of treated mice, with three to four mice per treatment (see Table 1). The epoxides were administered as three daily i.p. injections given between 9:00 and 10:00 a.m., at the doses noted in Table 1. Control mice received injections with the same volume of vehicle (1.0 mL/kg). On the morning following the last injection, the mice were killed by cervical dislocation, liver cytosol and microsomal fractions were prepared by differential centrifugation, and protein was determined using our automated modification of the Bradford procedure with bovine serum albumin as standard as previously described [23]. Tissue fractions were either assayed immediately or were stored at −80° for assay at a later time.

Enzyme assays. EH activities for CSO and TSO were determined in microsomal and cytosolic fractions (mEHcso, mEHtso, cEHcso, and cEHtso) using our previously described radiometric partition assay [23], where the tritiated epoxide is extracted with isooctane, and the remaining aqueous fraction assayed for diol formation by liquid scintillation counting. EH activity towards cholesterol-α-epoxide in microsomes (mEHchol) was determined from the rate of hydrolysis of [^{14}C]cholesterol-α-epoxide to its diol using TLC separation of ether extracts as

Table 1. Effects of epoxides on mouse liver weight and cell fraction protein

Treatment	Number of mice	Dose (mg/kg)	Relative liver weight (ratio to control)	Protein (ratio to control)	
				Microsomal	Cytosolic
Pesticides					
Heptachlor epoxide	4	25	1.27 ± 0.16*	1.82 ± 0.45*	1.12 ± 0.07*
Juvenoid R-20458	3	500	1.13 ± 0.06*	1.23 ± 0.18*	1.00 ± 0.13
Dieldrin	4	15	1.19 ± 0.08*	1.22 ± 0.15*	1.13 ± 0.06*
Tridiphane	4	500	2.13 ± 0.21*	1.91 ± 0.10*	0.90 ± 0.28
Natural products					
Disparlure	3	500	0.99 ± 0.10	1.17 ± 0.08	1.18 ± 0.06*
Limonin	3	100	1.09 ± 0.03	0.96 ± 0.18	0.95 ± 0.08
Nomilin	3	100	1.44 ± 0.15*	0.55 ± 0.11*	0.68 ± 0.06*
Epoxymethyloleate	3	500	1.09 ± 0.14	0.76 ± 0.20	0.87 ± 0.10*
Endogenous steroids					
Lanosterol epoxide	3	500	0.93 ± 0.05	0.64 ± 0.04*	0.78 ± 0.05
Cholesterol-α-epoxide	4	100	1.09 ± 0.03	0.92 ± 0.16	0.96 ± 0.09
Progesterone epoxide	4	100	1.06 ± 0.08	1.01 ± 0.11	1.01 ± 0.03
Industrial/Synthetics					
Epichlorohydrin	4	40	0.98 ± 0.04	0.94 ± 0.02	1.07 ± 0.05
Araldite	3	500	1.08 ± 0.03	1.04 ± 0.06	1.00 ± 0.04
trans-Stilbene oxide	3	100	1.13 ± 0.09*	1.35 ± 0.10	1.11 ± 0.02*
4'-Phenylchalcone oxide	3	200	1.01 ± 0.12	1.59 ± 0.19*	1.11 ± 0.04*

Values are the means ± SD for the number of mice noted, and are expressed as the ratio to the matched control mean. Representative control values are: relative liver weight, 5.57% of total body weight; microsomal protein, 10.8, and cytosolic protein, 87.0 mg/g liver.

* Significantly different from matched control, $P < 0.05$.

described by Sevanian and McLeod [24]. GST activities for CSO in microsomes (mGSTcso) and CSO and TSO in cytosol (cGSTcso and cGSTtso) were determined using our previously described radiometric partition assay [23] where the tissue is incubated in the presence of 5 mM glutathione, the diol and epoxide are extracted with hexanol, and the tritiated conjugate remaining in the aqueous fraction is detected by liquid scintillation counting. GST activities for CDNB in microsomes (mGSTcdnb), and CDNB, DCNB, and ENPP in cytosol (cGSTcdnb, cGSTdcnb, and cGSTenpp) were determined spectrophotometrically using our previously described modification [23] of the methods of Habig and Jakoby [25]. Carboxylesterase activities were measured as previously described [16]. In brief, carboxylesterase activities for MAL and CLOF in microsomes (mCEmal and mCEclof) were determined spectrophotometrically based on coupling of the hydrolysis to the reduction of *p*-iodonitrophenyltetrazolium by alcohol dehydrogenase. The hydrolysis of PNPA in microsomes (mCEpnpa) was assayed spectrophotometrically based upon the liberation of *p*-nitrophenol. Spectrophotometric assays of CE activity were performed using previously described conditions [16] with a Varian-Cary 219 UV/VIS spectrophotometer equipped with time drive and a temperature-controlled sample compartment, and interfaced with an Apple IIe computer. All assays were performed using protein concentrations (see the references cited above), which allow measurement on a linear portion of activity versus protein and activity versus time plots for the full range of activities encountered in this study. In addition, the EH assays were performed using substrate concentrations at 10 times the K_m, and these activities may be considered V_{max}. Other assays usually measure a number of isozymes, for which saturating concentrations are not available; however, substrate concentrations did approach solubility limits in most cases.

Statistical analysis. Tests for significant differences from matched control activities were performed using Student's two-tailed *t*-test with $P < 0.05$ as the criteria for significance. Correlations were performed essentially as described previously [9], but now employing a Lotus 1-2-3® spreadsheet. The values for individual teated animals were expressed relative to the average for the controls from the same batch. These data were filed in columns for each criteria measured, and then correlations between each group were determined.

RESULTS

The doses used for treatment were based upon either established non-toxic doses, preliminary tests which demonstrated near maximal non-lethal doses, or limited to 500 mg/kg. Over the 3 days of treatment, none of the epoxides caused significant weight loss as compared to controls (data not shown). Three days of treatment with a number of the epoxides resulted in significant, but for the most part, modest increases in the relative liver weights (Table 1). This was seen with all four pesticides tested, with tridiphane giving the greatest hepatotrophic response, and more modest responses following heptachlor epoxide, dieldrin and juvenoid R-20458. A single natural product, nomilin, and a single

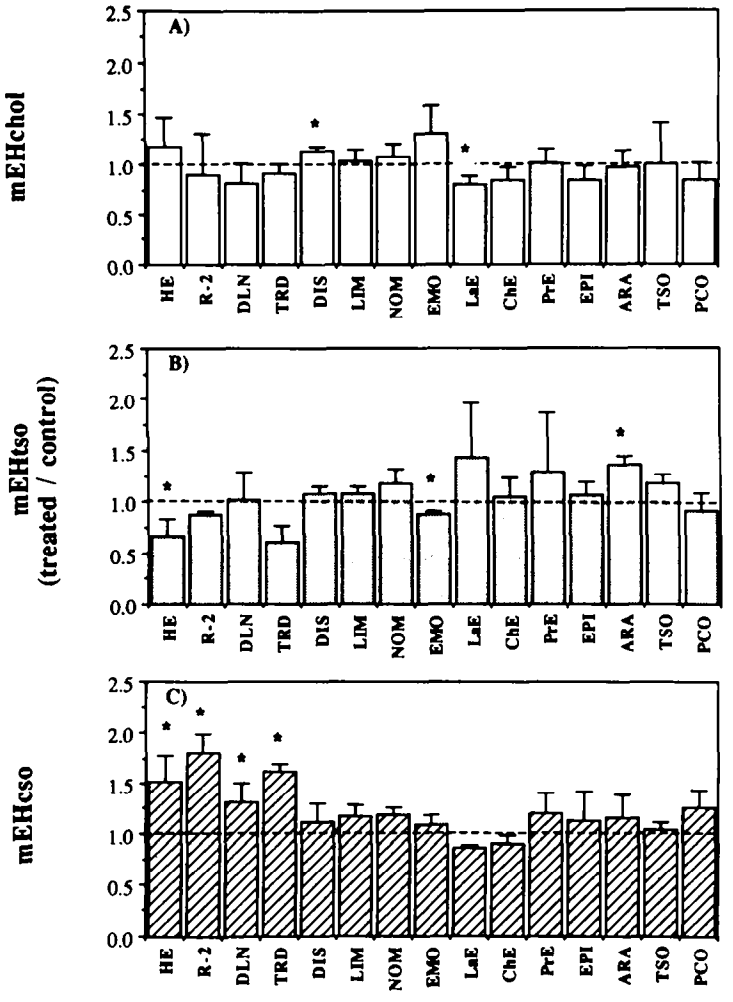

Fig. 2. Effects of epoxides on mouse liver microsomal epoxide hydrolase activities: (A) mEHchol, (B) mEHtso, and (C) mEHcso. Values are means ± SD for the number of mice noted in Table 1, and are expressed as the ratio to the matched control mean. Representative control values are: mEHchol, 0.20; mEHtso, 0.076; and mEHcso, 3.26 nmol/min/mg protein. The abbreviations used for the epoxides administered to mice are defined in the legend to Fig. 1. Key: (*) significantly different from matched control, $P < 0.05$.

synthetic epoxide, *trans*-stilbene oxide, also resulted in significant increases in relative liver weight. The increase in liver weight caused by nomilin appeared to be associated with gross liver damage.

Microsomal protein was increased significantly by all four pesticides (Table 1). 4′-Phenylchalcone oxide was the only other epoxide for which 3-day treatment resulted in a significant increase in microsomal protein. Mice treated with nomilin and lanosterol epoxide had a significant decrease in protein recovered in their microsomal fractions. Treatments with nomilin and with epoxymethyloleate also resulted in a significant decrease in cytosolic protein, while modest, but significant increases in cytosolic protein followed 3-day treatment with heptachlor epoxide, dieldrin, *trans*-stilbene oxide, and 4′-phenylchalcone oxide (Table 1).

Epoxide hydrolase activity in microsomes was studied for three substrates, mEHcso, mEHtso, and mEHchol (Fig. 2). mEHcso activity, which is selective for the "microsomal epoxide hydrolase," was increased significantly by all four of the pesticides, from 1.32-fold by dieldrin to 1.80-fold by juvenoid R-20458, but not by any of the other epoxides following 3 days of treatment. Except for a decrease in mEHtso after heptachlor epoxide, neither mEHtso nor mEHchol was affected significantly by the pesticides. mEHtso activity was increased slightly by araldite (1.35-fold) and decreased by epoxymethyloleate, while mEHchol activity was increased slightly by disparlure (1.14-fold), and decreased by lanosterol epoxide (Fig. 2). The hydrolysis of CSO in the cytosol (cEHcso) was also increased significantly by only the four pesticides. For two of these, tridiphane and heptachlor epoxide, the increases in activity in the cytosol of 3.05- and 2.39-fold, respectively, surpassed the comparative response in the microsomes (Fig. 3). cEHtso activity was increased by tridiphane treatment only. This finding was consistent with our previous findings that

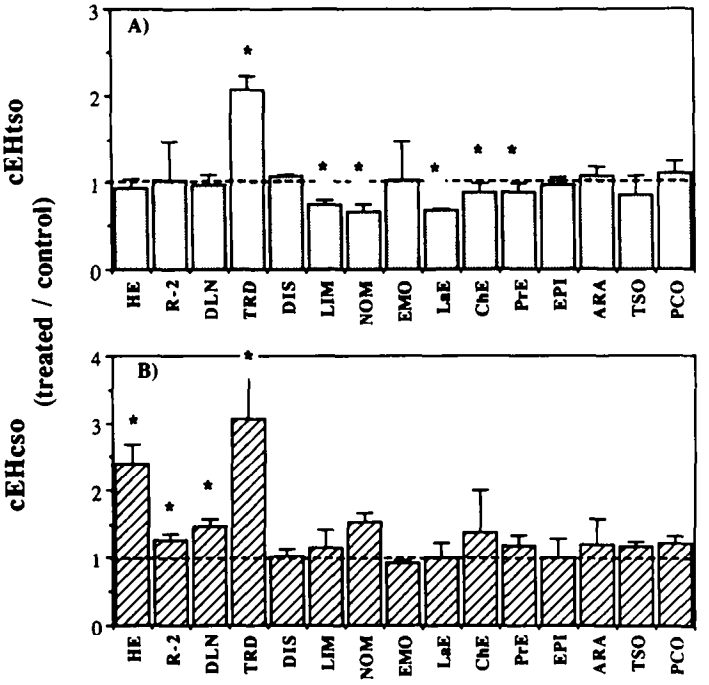

Fig. 3. Effects of epoxides on mouse liver cytosolic epoxide hydrolase activities: (A) cEHtso and (B) cEHcso. Values are means ± SD for the number of mice noted in Table 1, and are expressed as the ratio to the matched control mean. Representative control values are: cEHtso, 4.92; and cEHcso, 1.13 nmol/min/mg protein. The abbreviations used for the epoxides administered to mice are defined in the legend to Fig. 1. Key: (*) significantly different from matched control, $P < 0.05$.

tridiphane is a peroxisome proliferator, and that increases in cEHtso activity are characteristic of peroxisome proliferators [23]. A number of the epoxides caused significant decreases in cEHtso activity (Fig. 3).

The responses of the cytosolic GST activities to epoxide treatment were striking in the similarity of their responses to the pesticides. GST activities for three of the five substrates (cGSTtso, cGSTcso, and cGSTdcnb) were increased by all four pesticides with a similar ranking from greatest to least of: tridiphane, heptachlor epoxide, juvenoid R-20458, and dieldrin (Fig. 4). cGSTenpp activity was increased by three of the pesticides, but in this case, all were equipotent. cGSTcdnb activity was not affected significantly by the pesticides. Treatment with epoxides other than the pesticides, however, resulted in unique responses with the five GST substrates (Fig. 4). While some similarities were found in the responses of these five cytosolic GST activities, particularly in regard to pesticide treatment, it is obvious that each, with the possible exception of cGSTtso and cGSTdcnb, had its own selective response to epoxide treatment.

In our initial experiments with mGST activity, we tested all five substrates in control mouse liver microsomes. CDNB was the most active substrate detected by spectrophotometric assay (Fig. 5), with little, or no, detectable conjugation of ENPP or DCNB. CSO, however, produced low GST activity. This activity increased linearly with the addition of increasing amounts of microsomal protein (Fig. 5). When 120 μg/mL of protein was added, the cpm attributable to GST activity [i.e total cpm − background cpm (true background plus nonenzymatic conjugation)] equaled that of total background cpm. A similar protein concentration was subsequently used for all mGSTcso assays. We were unable to detect activity with TSO as substrate. As TSO would behave identically to CSO during hexanol extraction of the epoxides and diols from the assay mixture, this apparently rules out incomplete extraction as the mechanism of radioactivity being retained in the aqueous phase.

Each substrate, mGSTcdnb and mGSTcso, gave a unique pattern of responses to the epoxide treatments. mGSTcso activity was increased significantly by nomilin, epoxymethyloleate and lanosterol epoxide, and decreased by araldite treatment. mGSTcdnb activity, in contrast was increased significantly by araldite, *trans*-stilbene oxide, and 4′-phenylchalcone oxide, and decreased by nomilin treatment (Fig. 6). Besides differing from each other, neither of the microsomal GST activities demonstrated a pattern of induction similar to any of their cytosolic counterparts.

The three microsomal carboxylesterase activities monitored, mCEmal, mCEclof, and mCEpnpa, also demonstrated apparent substrate specific responses to the epoxide treatments (Fig. 7). The only consistent changes were an increase in all three activities following treatment with heptachlor

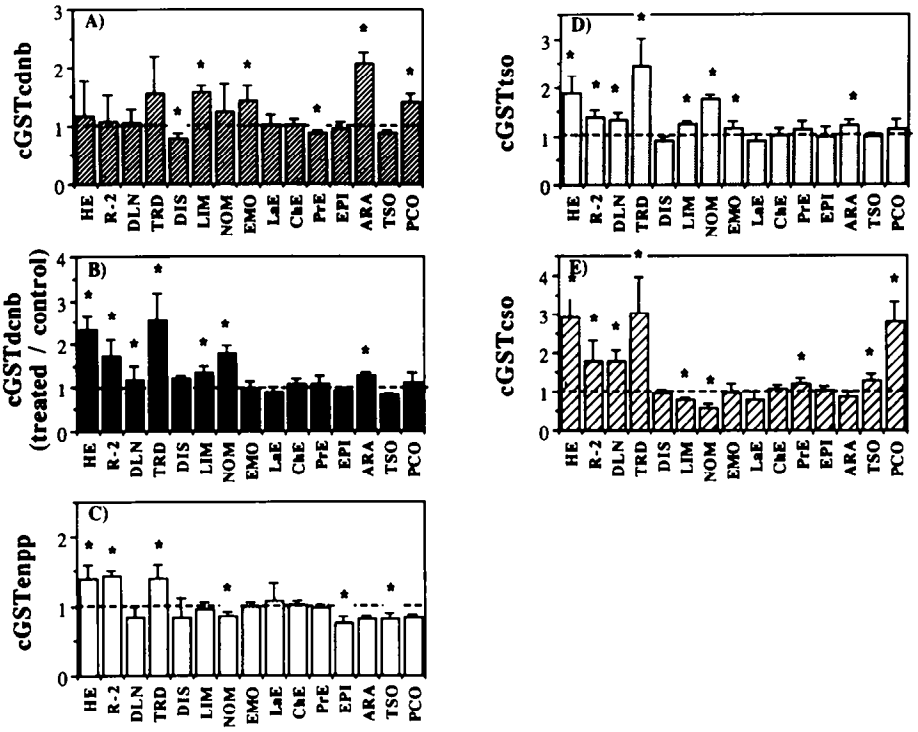

Fig. 4. Effects of epoxides on mouse liver cytosolic glutathione *S*-transferase activities: (A) cGSTcdnb, (B) cGSTdcnb, (C) cGSTenpp, (D) cGSTtso, and (E) cGSTcso. Values are means ± SD for the number of mice noted in Table 1, and are expressed as the ratio to the matched control mean. Representative control values are: cGSTcdnb, 2.12 μmol/min/mg protein; cGSTdcnb, 18.9; cGSTenpp, 80.8; cGSTtso, 6.2; and cGSTcso, 33.5 nmol/min/mg protein. The abbreviations used for the epoxides administered to mice are defined in the legend to Fig. 1. Key: (*) significantly different from matched control, $P < 0.05$.

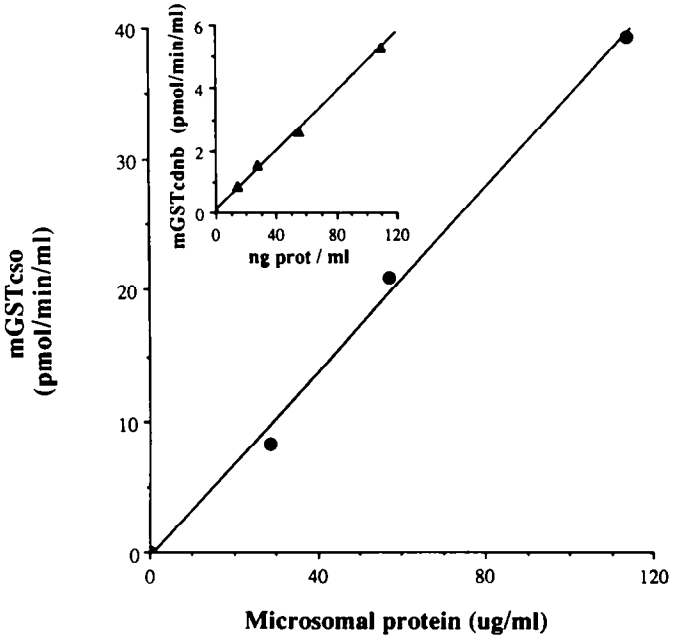

Fig. 5. Glutathione-dependent conjugation in mouse liver microsomes. The increase in mGSTcso activity (●), as measured by the radiometric partition assay, with addition of increasing amounts of microsomal protein is shown. In the inset, mGSTcdnb activity (▲), as measured by spectrophotometric assay, is shown for comparative purposes. The results are the means of triplicate assays from two control mouse liver cytosols.

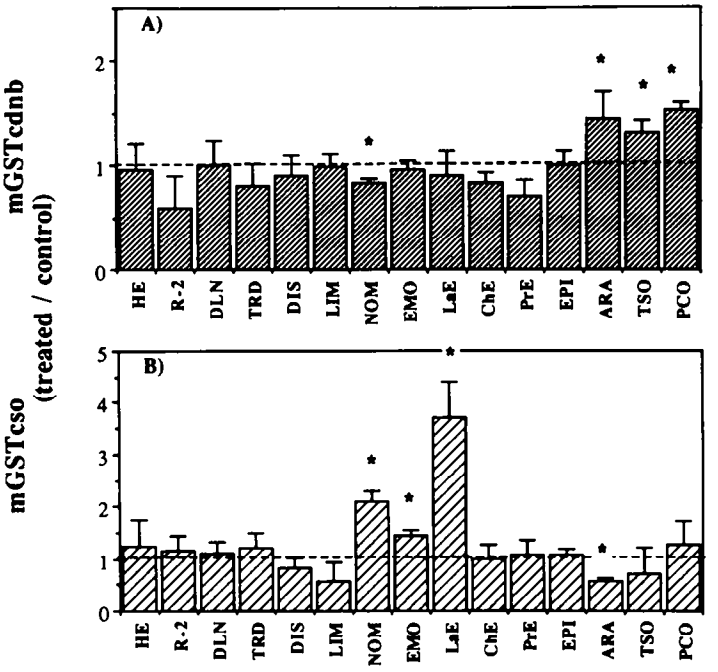

Fig. 6. Effects of epoxides on mouse liver microsomal glutathione *S*-transferase activities: (A) mGSTcdnb and (B) mGSTcso. Values are means ± SD for the number of mice noted in Table 1, and are expressed as the ratio to the matched control mean. Representative control values are: mGSTcdnb, 76.6; and mGSTcso, 0.18 nmol/min/mg protein. The abbreviations used for the epoxides administered to mice are defined in the legend to Fig. 1. Key: (*) significantly different from matched control, $P < 0.05$.

epoxide and decreases in all three following treatment with lanosterol epoxide. mCEmal activity was affected by only two other epoxides, being increased by araldite and decreased by limonin. mCEclof activity was increased by three and decreased by three additional epoxides, while mCEpnpa activity was increased by four and decreased by one additional epoxide (Fig. 7).

Casual observation of the comparative responses of the various cell fractions and enzyme activities to the epoxide treatments suggests that certain of these are altered in a similar fashion. This can be expressed quantitatively by searching for correlations among the responses to treatment. The changes in liver weight, cell fraction protein, or enzyme activity for each mouse were expressed relative to the respective mean value of the corresponding controls, and used to determine correlation coefficients. These comparisons can be observed graphically. The scatter plot for the best correlation found, cGSTdcnb vs cGSTtso, $r = 0.89$, is presented in Fig. 8. With measurements of liver weight, protein in two cell fractions, and 15 enzyme activities, this results in a total of 153 correlations (Table 2). These will not be discussed individually, but the top ten correlations are highlighted in Table 2.

Of the top 10 correlations found in this comparison, four were with the response in liver weight, and two each with the responses in microsomal protein, cEH and cGST activities (Table 2). The correlations between GST activities for different substrates could easily arise from cross-specificity of the GST isozymes for these substrates. What is surprising, however, is that the high correlations with relative liver weight and microsomal protein occurred with cytosolic enzyme activities. In this regard, particularly high correlations were found between the relative liver weight and cGSTtso, $r = 0.75$, and cEHcso, $r = 0.62$, and between microsomal protein and cEHcso, $r = 0.62$. These studies, therefore, not only provide data on the response of mouse liver epoxide-metabolizing enzymes to acute treatment with environmentally encountered epoxides, but also offer directions for study on the relationship between liver growth and cytosolic enzymes.

DISCUSSION

The major purpose of this study was to test if environmentally encountered epoxides affect the activity of mouse liver enzymes which could be involved in epoxide metabolism. Towards this purpose, EH and GST activities in mouse liver microsomes and cytosol were measured after 3 days of treatment with 15 different epoxides. This protocol also was utilized to further elucidate the response of microsomal carboxylesterase activities to xenobi-

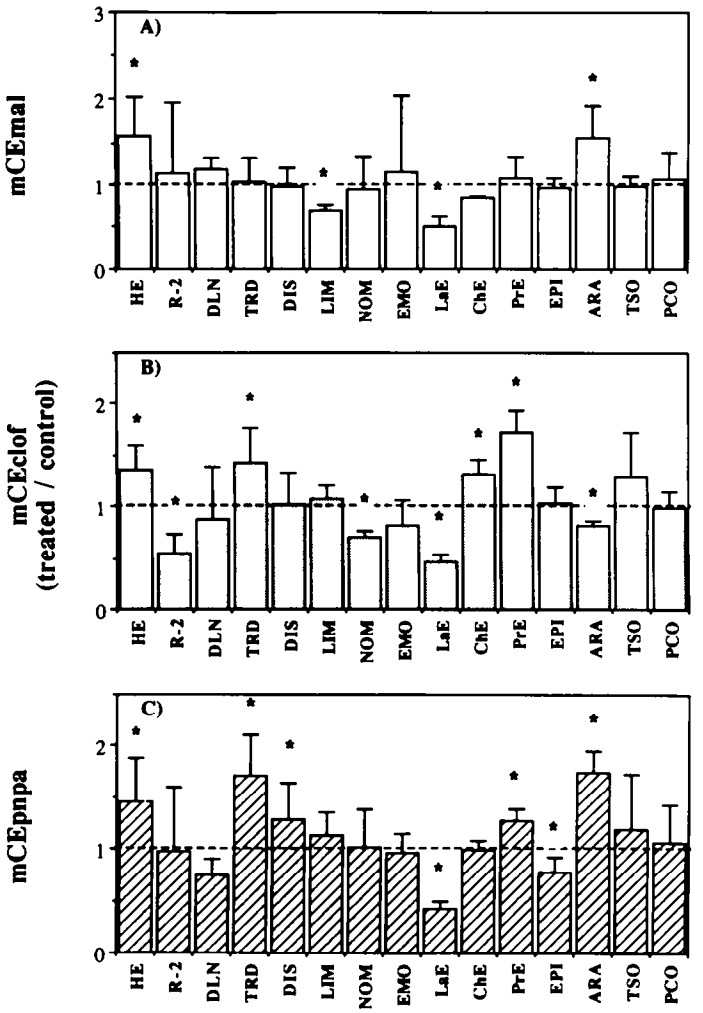

Fig. 7. Effects of epoxides on mouse liver microsomal carboxylesterase activities: (A) mCEmal, (B) mCEclof, and (C) mCEpnpa. Values are means ± SD for the number of mice noted in Table 1, and are expressed as the ratio to the matched control mean. Representative control values are: mCEmal, 25.1; and mCEclof, 85.5 nmol/min/mg protein; and mCEpnpa, 1.09 μmol/min/mg protein. The abbreviations used for the epoxides administered to mice are defined in the legend to Fig. 1. Key: (*) significantly different from matched control, $P < 0.05$.

otics. Finally, the data provided were subjected to further analysis to assess whether there existed any strong correlations in the response of liver compartments to a number of distinct xenobiotic treatments.

When the responses of the hepatic components as a whole are taken into consideration, they may offer some information on the bioactivity of the epoxides in mammalian systems. The epoxides utilized have been classified based upon their occurrence: pesticides, natural, etc. Due to the structural diversity of these compounds, even within a group, this classification does not provide for SAR analysis between categories. These categories, however, do reflect environmental occurrences of the epoxides, and specific differences in the enzymatic responses to the epoxides within different classes were seen, as follows. The pesticide epoxides, were, in general, very inductive compounds. Of the fifteen parameters measured, there were significant increases in twelve, eleven, eight and eight by heptachlor epoxide, tridiphane, juvenoid R-20458, and dieldrin, respectively. With the exclusion of epichlorohydrin which did not increase any of these parameters, the other synthetic epoxides ranked next in this regard. In contrast, none of the natural or endogenous epoxides caused increases in more than four parameters. The natural and endogenous epoxides seemed more prone to cause decreases in the parameters measured. This was particularly true for nomilin and lanosterol epoxide which decreased seven and five parameters, respectively. This points out the relative activity of these compounds pertaining to their inductive or inhibitory effect on these enzyme activities.

The induction of these enzyme activities is not an immediate indication of toxicity. Alterations in the

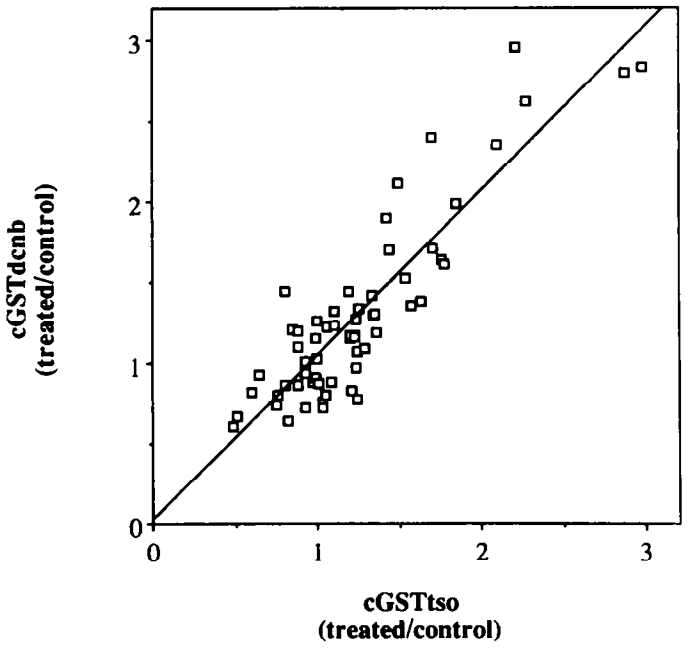

Fig. 8. Representative scatter plot of changes in cGSTdcnb and cGSTtso activities in individual mice. Values for the two enzyme activities were first expressed relative to the mean of the appropriate matched controls, and then plotted. The correlation coefficients shown in Table 2 were determined in a similar manner except that a different software program was used to facilitate calculations. The equation for the above plot is $y = 0.018 + 1.021x$, with $r = 0.89$.

Table 2. Correlations among the relative responses of liver weight, protein, and enzyme activities to environmental epoxide treatment

	Relative liver weight	mPROT	cPROT	mEH			cEH		mGST		cGST					mCE	
				cso	chol	tso	tso	cso	cdnb	cso	cdnb	dcnb	enpp	cso	tso	mal	clof
mPROT	0.538	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
cPROT	−0.115	0.502	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mEHcso	0.440	0.466	0.166	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mEHchol	−0.358	−0.508	−0.238	−0.169	—	—	—	—	—	—	—	—	—	—	—	—	—
mEHtso	0.028	0.077	0.274	0.276	0.136	—	—	—	—	—	—	—	—	—	—	—	—
cEHcso	0.625	0.616	0.338	0.488	−0.321	0.390	—	—	—	—	—	—	—	—	—	—	—
cEHtso	0.733	0.591	−0.106	0.518	−0.449	0.041	0.537	—	—	—	—	—	—	—	—	—	—
mGSTcdnb	−0.217	0.071	0.146	−0.186	−0.004	−0.123	−0.151	−0.141	—	—	—	—	—	—	—	—	—
mGSTcso	−0.122	−0.411	−0.468	−0.207	0.475	0.012	−0.263	−0.251	0.003	—	—	—	—	—	—	—	—
cGSTcdnb	0.184	−0.026	−0.469	0.188	0.012	0.038	0.066	0.187	0.065	0.247	—	—	—	—	—	—	—
cGSTdcnb	0.662	0.470	−0.215	0.512	−0.412	−0.061	0.274	0.726	−0.234	−0.148	0.257	—	—	—	—	—	—
cGSTenpp	0.342	0.338	−0.280	0.442	−0.110	−0.040	0.162	0.535	−0.321	0.105	0.308	0.626	—	—	—	—	—
cGSTcso	0.448	0.729	0.214	0.475	0.342	−0.196	0.353	0.608	0.117	−0.100	−0.020	0.553	0.460	—	—	—	—
cGSTtso	0.750	0.499	−0.280	0.500	−0.448	−0.156	0.314	0.799	−0.119	−0.207	0.203	0.889	0.512	0.563	—	—	—
mCEmal	0.042	0.303	0.268	0.271	0.045	0.135	0.075	0.195	0.263	−0.168	−0.102	0.095	0.069	0.267	0.112	—	—
mCEclof	0.213	0.369	0.239	0.215	−0.198	0.270	0.319	0.340	−0.038	−0.333	0.060	0.066	0.006	0.171	0.137	0.220	—
mCEpnpa	0.329	0.415	0.182	0.385	−0.201	0.314	0.430	0.514	0.174	−0.500	−0.004	0.296	0.149	0.233	0.336	0.477	0.471

Correlation coefficients were calculated from a comparison of values from individual treated mice expressed as a ratio to the mean value of appropriately matched controls; N = 61, and for all r values >0.330 the correlation is significantly greater than zero with $P < 0.01$. The ten highest correlation coefficients have been outlined.

activity of xenobiotic-metabolizing enzymes can alter the metabolism of other xenobiotic or endogenous compounds (xenobiotic–xenobiotic interactions). Whether this is deleterious, or not, depends on the specific isozymes induced, or inhibited, and the other compound in question (see Ref. 26 for a recent discussion). Even during cocarcinogenesis, a single inducing agent may activate one carcinogen, while inhibiting another [27]. The cellular responses which accompany the induction of xenobiotic enzyme activities, especially those associated with peroxisome proliferation, have however, been associated with nongenotoxic carcinogenesis and/or tumor promotion [28, 29].

Among the EHs studied, the inductive response of mEHcso (i.e. the microsomal epoxide hydrolase)

to a number of xenobiotics has been well documented [10, 12, 15]. The mechanism of this induction has not been ascertained yet, but it does not appear to be related to induction of self-metabolism. First, many compounds which are not epoxides, nor apparently metabolized to epoxides, induce mEH [10]. Furthermore, in this study araldite, which is a good substrate for mEH [30], had no effect, while tridiphane, which is only very slowly turned-over by mEH [31], increased the activity of mEH.

The EH activities offer a good opportunity to analyze the power of the biological correlations in response to xenobiotic treatment. Of the five activities measured, mEHcso activity has been distinguished from mEHtso [32], cEHtso [33], and mEHchol [34] by immunochemical means. Furthermore, cEHtso has been partially separated from cEHcso by affinity chromatography [35]. In contrast, some evidence exists for a similar origin of mEHcso and cEHcso, arising in part from alterations in the endoplasmic reticulum, through either chemico-biological changes to the membrane or mechanical alterations during cell fractionation [36]. The highest correlations among the five EHs were cEHtso with cEHcso, mEHcso with cEHtso, and mEHcso with cEHcso at 0.54, 0.52, and 0.49, respectively. In a previous correlated study which compared the responses of EHs in liver, kidney, and testes of control and clofibrate-treated mice, the corresponding correlations were 0.41, 0.73, and 0.51 [9]. A uniformity in changes in the membrane environment by one xenobiotic, clofibrate, as opposed to diverse response from the fifteen epoxides employed may explain the lower correlation found between cEHcso and mEHcso in this study.

Unless a correlation is drawn between two selective activities for the same isozyme (e.g. the correlation between mEHcso and mEHbpo in the preceding study was 0.96), a number of variables can produce, or affect, an apparent correlation. The two most obvious variables are first, when a single isozyme has only partial specificity for the two substrates, the response of other isozymes utilizing this substrate will decrease the correlation. Second, however, when two distinct enzymes (or isozymes) share regulatory control, apparent correlations will increase.

The latter factor, shared regulatory control, may explain why the highest correlations of EHs for another activity were cEHcso with cGSTtso, cGSTdcnb, and cGSTenpp with coefficients of 0.80, 0.73 and 0.54, respectively. Diol formation from CSO in the cytosol (i.e. EH activity) has been demonstrated by GLC in mice [14] and TLC in rats [9], ruling out the possibility that the product formation identified here was actually a glutathione conjugate formed from glutathione retained in the cytosolic fraction. However, since the structural protein associated with cEHcso activity has not been purified and characterized yet, we cannot rule out the possibility that this enzyme may share hydrolytic and glutathione conjugative activity. cGSTtso, in turn, had a very high correlation with cGSTdcnb. In this case it is just as, if not more, likely that this correlation arose from shared isozyme selectivity for the two substrates. The isozyme selectivity for glutathione conjugation of TSO or CSO has not been studied thoroughly. That the current data suggest that TSO may be conjugated by mouse liver isozymes which are more selective for DCNB is consistent with a report on mouse liver cGSTtso activity [6]. Lee *et al.* [5] have shown that one of three major mouse liver cytosolic isozymes has relatively high activity for DCNB. More recently, Benson *et al.* [37] have shown high DCNB activity associated with three of seven isozymes isolated from female mice induced with butylhydroxyanisole, specifically those containing Yb1 subunits.

The mGST has been found previously to be under separate regulatory control from cGST based upon xenobiotic induction [38, 39]; thus, it is not surprising that a low correlation was found between the two mGST activities and the cGST activities. Nor was there a good correlation between the mGSTcso and mGSTcdnb activities. Activation of mGST activity does occur [40, 41], and in rat liver microsomes it has been shown to be selective for those substrates with relatively low nonenzymatic conjugative activity [40]. It is possible, therefore, that the lack of correlation between mGSTcdnb and mGSTcso activities may be due to differential activation of the activities. However, the possibility of a second mGST isozyme should also be considered.

We have now described apparent mGSTcso activity in rat and mouse liver microsomes ([23] and current study), and in both cases have found differential responses to xenobiotics which either increase or decrease one of the activities. At this time, we have not confirmed the structural identity of the product as a glutathione conjugate. Supposition of its occurrence depends upon its formation being glutathione dependent (i.e. radiolabeled product was only formed when glutathione was added to the assay system), and its selective resistance to extraction by hexanol, which is characteristic of glutathione conjugates with stilbene oxides. While these data do not rule out the formation of some other non-diol product, they are highly suggestive of glutathione conjugate formation. Definitive elucidation of product formation awaits further study. Furthermore, under the conditions of the radiometric partition assay employed to measure mGSTcso activity, it is possible that epoxide formed by mEHcso may not have been totally extracted with hexanol. This could lead to an apparent mGSTcso activity which would increase with increased microsomal protein used. As mEHcso activity is much higher than mEHtso, this would explain why no comparable activity was seen for mGSTtso even though the geometric isomers have identical extraction efficiencies. However, it this was the case, one would expect a relatively high correlation between the responses of mGSTcso and mEHcso, whereas the correlation coefficient found was −0.207. Also extensive tests have indicated that a variety of agents including high lipid, high protein, high salt and high detergent fail to perturb the published partition characteristics for the stilbene oxides and their metabolites. Morgenstern *et al.* [40] found no activity for CSO conjugation in a purified rat liver mGST. This suggests that the mGSTcso activity observed in this and the previous study arose

either from a separate isozyme, or from cytosolic contamination of the microsomal preparation. If the latter case was true, however, we would have expected a fairly high correlation between cGSTcso and mGSTcso, rather than the $r = -0.10$ found.

Among the three CE activities studied, we were able to identify additional xenobiotics which are capable of inducing the activity of these enzymes. The increase in mCEpnpa and mCEclof by the peroxisome proliferator tridiphane was similar in magnitude to our previously observed increases in these activities by clofibrate. However, clofibrate increased mCEmal activity also, while tridiphane had no effect on this activity [16]. This suggests that the response of mCE activities to peroxisome proliferators is not entirely uniform. Of the various enzymatic activities studied, the mCEs were the only ones that were increased following administration of cholesterol and progesterone epoxides. While no strong correlation was noted among the three mCE activities, the response of mCEpnpa correlated almost equally with that of mCEmal and mCEclof, whereas the response of the latter two was only weakly correlated.

These studies have shown, therefore, that there exists a wide variation in the magnitude and direction of response of xenobiotic-metabolizing enzymes to environmentally encountered epoxides. Those epoxides used as pesticides, however, do seem to have a relatively strong inductive effect on these activities. Further, a fairly strong correlation exists between the increases in liver size and increases in certain cytosolic enzyme activities. Our observations on the correlation between cGST activities and the lack of correlation between two mGST activities may provide insights which suggest further studies concerning the isozyme selectivity of cGSTs for stilbene epoxides and the possibility of an additional isozyme of mGST.

Acknowledgements—This study was supported in part by US Public Health Service Grants DA05102 (D.E.M.) and ES0271 (B.D.H.). B.D.H. is the recipient of a Burroughs-Wellcome Toxicology Scholar Award.

REFERENCES

1. Manson MM, Epoxides—Is there a human health problem? *Br J Ind Med* **37**: 317–336, 1980.
2. Jerina DM and Daly JW, Arene oxides: A new aspect of drug metabolism. *Science* **185**: 573–582, 1974.
3. Hemminki K, Falck K and Vanio H, Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals; Epoxides, glycidyl ethers, methylating and ethylating agents, halogenated hydrocarbons, hydrazine derivatives, aldehydes, thiuram and dithiocarbamate derivatives. *Arch Toxicol* **46**: 277–285, 1980.
4. Canter DA, Zeiger E, Haworth S, Lawlor T, Mortelmans K and Speck W, Comparative mutagenicity of aliphatic epoxides in Salmonella. *Mutat Res* **172**: 105–138, 1986.
5. Lee C-Y, Johnson L, Cox RH, McKinney JD and Lee S-M, Mouse liver glutathione *S*-transferases. Biochemical and immunological characterization. *J Biol Chem* **256**: 8110–8116, 1981.
6. Mannervik B and Danielson UH, Glutathione transferases—Structure and catalytic activity. *CRC Crit Rev Biochem* **23**: 283–337, 1988.
7. Boyer TD, The glutathione *S*-transferases: An update. *Hepatology* **9**: 486–496, 1989.
8. Morgenstern R, Lundquist G, Andersson G, Balk L and DePierre JW, The distribution of microsomal glutathione transferase among different organelles, different organs, and different organisms. *Biochem Pharmacol* **33**: 3609–3614, 1984.
9. Moody DE, Silva MH and Hammock BD, 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* **35**: 2073–2080, 1986.
10. Wixtrom RN and Hammock BD, Membrane-bound and soluble-fraction epoxide hydrolases. *Biochem Pharmacol Toxicol* **1**: 1–93, 1985.
11. Meijer J and DePierre JW, Cytosolic epoxide hydrolase. *Chem Biol Interact* **64**: 207–249, 1988.
12. Thomas H and Oesch F, Functions of epoxide hydrolases. *ISI Atlas Sci: Biochem* **1**: 287–291, 1988.
13. Oesch F and Golan M, Specificity of mouse liver cytosolic epoxide hydrolase for K-region epoxides derived from polycyclic aromatic hydrocarbons. *Cancer Lett* **9**: 169–175, 1980.
14. Hammock BD and Hasagawa LS, Differential substrate selectivity of murine hepatic cytosolic and microsomal epoxide hydrolases. *Biochem Pharmacol* **32**: 1155–1164, 1983.
15. Schmassmann H, Sparrow A, Platt K and Oesch F, Epoxide hydratase and benzo(a)pyrene monooxygenase activities in liver, kidney and lung after treatment of rats with epoxides of widely varying structures. *Biochem Pharmacol* **27**: 2237–2245, 1978.
16. Ashour M-BA, Moody DE and Hammock BD, Apparent induction of microsomal carboxylesterase activities in tissues of clofibrate-fed mice and rats. *Toxicol Appl Pharmacol* **89**: 361–369, 1987.
17. Satoh T, Role of carboxylesterases in xenobiotic metabolism. *Rev Biochem Toxicol* **8**: 155–181, 1987.
18. Lewis DA and McGhie JK, Isolation and reactions of lanost-8:24-dien-3-β-ol. *Chem Ind* pp. 550–551, 1956.
19. Mumby SM and Hammock BD, Stability of epoxide-containing juvenoids to dilute aqueous acid. *J Agric Fed Chem* **27**: 1223–1228, 1979.
20. Gill SS and Hammock BD, Distribution and properties of a mammalian soluble epoxide hydrase. *Biochem Pharmacol* **29**: 389–395, 1980.
21. Mullin CA and Hammock BD, Chalcone oxides—Potent selective inhibitors of cytosolic epoxide hydrolase. *Arch Biochem Biophys* **216**: 423–439, 1982.
22. Gill SS, Ota K and Hammock BD, Radiometric assays for mammalian epoxide hydrolases and glutathione *S*-transferase. *Anal Biochem* **131**: 273–282, 1983.
23. Moody DE and Hammock BD, 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* **89**: 37–48, 1987.
24. Sevanian A and McLeod LL, Catalytic properties and inhibition of hepatic cholesterol-epoxide hydrolase. *J Biol Chem* **261**: 54–59, 1986.
25. Habig WH and Jakoby WB, Assays for differentiation of glutathione *S*-transferases. *Methods Enzymol* **77**: 398–405, 1981.
26. Siest G, Batt AM, Fournel-Gigleux, Galteau MM, Wellman-Bednawska M, Minn A and Costesec A, Induction of plasma and tissue enzymes by drugs: Significance in toxicological studies. *Xenobiotica* **18** (Suppl 1): 21–34, 1988.
27. Williams GM, Modulation of chemical carcinogenesis by xenobiotics. *Fundam Appl Toxicol* **4**: 325–344, 1984.
28. Schulte-Hermann R, Adaptive liver growth induced by

xenobiotic compounds: Its nature and mechanism. *Arch Toxicol* **Suppl 2**: 113–124, 1979.
29. Reddy JK and Lalwani ND, Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit Rev Toxicol* **12**: 1–58, 1983.
30. Bentley P, Bieri F, Kuster H, Muakkassah-Kelly S, Sagelsdorff P, Stäubli W and Waechter F, Hydrolysis of bisphenol A diglycidylether by epoxide hydrolases in cytosolic and microsomal fractions of mouse liver and skin: Inhibition by bis epoxycyclopentylether and the effects upon the covalent binding to mouse skin DNA. *Carcinogenesis* **10**: 321–327, 1989.
31. Magdalou J and Hammock BD, Metabolism of tridiphane (2-(3,5-dichlorophenyl)-2(2,2,2-trichloroethyl)oxirane) by hepatic epoxide hydrolases and glutathione *S*-transferases in mouse. *Toxicol Appl Pharmacol* **91**: 439–449, 1987.
32. Guenthner TM and Oesch F, Identification and characterization of a new epoxide hydrolase from mouse liver microsomes. *J Biol Chem* **258**: 15054–15061, 1983.
33. Guenthner TM, Hammock BD, Vogel U and Oesch F, Cytosolic and microsomal epoxide hydrolases are immunologically distinguishable from each other in rat and mouse. *J Biol Chem* **256**: 3163–3166, 1981.
34. Levin W, Michaud DP, Thomas PE and Jerina DM, Distinct rat hepatic microsomal epoxide hydrolases catalyze the hydration of cholesterol 5,6α-oxide and certain xenobiotic alkene and arene oxides. *Arch Biochem Biophys* **220**: 485–494, 1983.
35. Hammock BD, Prestwich GD, Loury DN, Cheung PYK, Eng W-S, Park S-K, Moody DE, Silva MH and Wixtrom RN, Comparison of crude and affinity purified cytosolic epoxide hydrolases from hepatic tissue of control and clofibrate-fed mice. *Arch Biochem Biophys* **244**: 292–309, 1986.
36. Kizer DE, Clouse JA, Ringer DP, Hanson-Painton O, Vaz AD, Palakodety RB and Griffin MJ, Assessment of rat liver microsomal epoxide hydrolase as a marker of hepatocarcinogenesis. *Biochem Pharmacol* **34**: 1795–1800, 1985.
37. Benson AM, Hunkeler MJ and York JL, Mouse hepatic glutathione transferase isoenzymes and their differential induction by anticarcinogens: Specificities of butylated hydroxyanisole and bisethylxanthogen as inducers of glutathione transferases in male and female CD-1 mice. *Biochem J* **261**: 1023–1029, 1989.
38. Morgenstern R, Meijer J, DePierre JW and Ernster L, Characterization of rat-liver microsomal glutathione *S*-transferase activity. *Eur J Biochem* **104**: 167–174, 1980.
39. Mukhtar H, Baars AJ and Breimer DD, Differences in inducibility of particulate and cytosolic rat liver glutathione *S*-transferase activities. *Xenobiotica* **11**: 367–371, 1981.
40. Morgenstern R, Lundqvist G, Hancock V and DePierre JW, Studies on the activity and activation of rat liver microsomal glutathione transferase, in particular with a substrate analogue series. *J Biol Chem* **263**: 6671–6675, 1988.
41. Andersson C, Soderstrom M and Mannervik B, Activation and inhibition of microsomal glutathione transferase from mouse liver. *Biochem J* **249**: 819–823, 1988.