Biochemical Pharmacology, Vol. 55, pp. 1059–1069, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00679-5

Effects of Chemical Inducers on Human Microsomal Epoxide Hydrolase in Primary Hepatocyte Cultures

*Christopher Hassett, Elizabeth M. Laurenzana, Jaspreet S. Sidhu and Curtis J. Omiecinski**

Department of Environmental Health, University of Washington, Seattle, WA 98105-6099, U.S.A.

ABSTRACT. Human microsomal epoxide hydrolase (mEH; EC 3.3.2.3) is an important biotransformation enzyme and potential risk determinant for pathologies such as cancer and teratogenesis. Currently, the effects of chemical exposures on human mEH gene expression are largely unknown, but they may constitute a unique modifier of disease susceptibility. To examine this issue, we exposed cultures of primary human hepatocytes isolated from seven donors to prototypic chemical inducers [such as phenobarbital (PB), polyaromatic hydrocarbons, dexamethasone, butylated hydroxyanisole, and ciprofibrate]. Basal levels of mEH RNA and protein were detected readily in untreated cells. Chemical treatment of cultured hepatocytes resulted in variable mEH RNA and protein expression, but, in general, only modest modulatory effects were detected following these exposures. The maximum increase in mEH RNA expression observed was approximately 3.5-fold following Arochlor 1254 exposure. Immunochemical levels of mEH protein were quantified for all treatment groups in three cultures and demonstrated less overall variation and, in general, a lack of concordance with corresponding mEH RNA levels. Cytochrome P450 (CYP) 1A2 and 3A mRNA levels were measured before and following exposure to β-naphthaflavone and PB, respectively, to permit independent evaluation of hepatocyte inducer responsiveness. Substantial increases in RNA expression levels for both the *CYP1A2* and *CYP3A* genes demonstrated that the hepatocyte cultures were robust and highly responsive to inducer treatment. These results indicate that the mEH gene in human hepatocytes is only modestly responsive to chemical exposures. BIOCHEM PHARMACOL **55**;7:1059–1069, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. microsomal epoxide hydrolase; human; primary hepatocytes; cell culture

Biotransformation of endogenous and foreign chemical compounds is accomplished principally by enzymes encoded by phase I and II genes. Experiments, particularly with animal models, have demonstrated that xenobiotic exposures often modulate basal expression patterns of xenobiotic-metabolizing genes. Induction of specific biotransformation enzymatic activities can thereby alter the disposition and toxicologic fate of an exposed substance. However, predicting genetic effects of chemical exposures in humans based on extrapolation from animal experiments can be problematic, due to the existence of species differences in biotransformation enzyme profiles [1, 2], species-specific gene induction idiosyncrasies [3, 4], and the large number of genetic polymorphisms present in outbred human populations [5, 6] relative to experimental organisms.

In vitro methods offer a valuable surrogate to whole animal studies. In particular, primary cultures of rodent hepatocytes maintained under well-defined experimental conditions have shown great promise in reproducing gene induction effects observed in animal studies [7, 8]. Our laboratory has developed methods to culture primary rat hepatocytes, which preserve a highly differentiated phenotype and *in vivo* response characteristics following exposure to prototypical chemical inducers of xenobiotic-metabolizing genes [9–11]. Improvements in isolation and culturing methodologies now enable the use of primary human hepatocytes as a viable means for predicting chemical exposure effects in humans [12–15].

Xenobiotic-metabolizing mEH† is a member of a group of enzymes (EC 3.3.2.3) that includes soluble EH, leukotriene A_4 hydrolase, cholesterol epoxide hydrolase, and hepoxilin hydrolase [16]. Among these enzymes, mEH is prominent with respect to its broad capacity to hydrolyze foreign chemical-derived epoxides. Striking structural similarities across mammalian mEH proteins have been deduced from cDNA sequence information [17–19], which suggest an important endogenous role for mEH apart from xenobiotic metabolism. Studies have indicated a role for mEH in bile acid transport [20–22], although conflicting data have been reported [23].

Evidence has accumulated supporting an important role for mEH in biotransformation reactions and its potential

* Corresponding author: Dr. Curtis J. Omiecinski, Department of Environmental Health, University of Washington, 4225 Roosevelt Way N.E. #100, Seattle, WA 98105-6099. Tel. (206) 543-1700; FAX (206) 685-4696; E-mail: cjo@u.washington.edu.

† *Abbreviations:* mEH, microsomal epoxide hydrolase; PB, phenobarbital; β-NF, beta-naphthaflavone; DEX, dexamethasone; ARO, Aroclor 1254; BHA, butylated hydroxyanisole; CIPRO, ciprofibrate; PAH, polyaromatic hydrocarbons; and CYP, cytochrome P450.

Received 1 August 1997; accepted 7 November 1997.

involvement in several pathologic disease processes. For example, teratogenic risk associated with mEH levels and anticonvulsant chemical exposure has been implicated in animal [24] and human [25] studies. The enzyme also participates in the generation of carcinogenic metabolites of polyaromatic hydrocarbons [26–28]. Polymorphic amino acids in the human mEH protein have been identified [29, 30], and specific allelic variants were reported to be over-represented in cases of hepatocellular carcinoma [31] and ovarian cancer [32].

The structure [33], expression [34–37], and polymorphic amino acids [29] of human mEH have been characterized in our laboratory to facilitate understanding the importance of this enzyme in toxicities. An additional risk factor for chemically induced toxicities may be the uncoordinated regulation of genes that generate reactive metabolites, relative to those that detoxify bioactivated agents. Primary human hepatocytes offer an effective means to test this hypothesis with respect to mEH.

In this investigation, we used primary human hepatocytes to examine the responsiveness of the mEH gene to an array of prototypic chemical inducing agents. Although cytochrome P450 marker genes were highly responsive to chemical treatment in cultures established from seven donors, human mEH expression was affected only modestly by these agents. This work is the first characterization of mEH gene chemical responsiveness in primary human hepatocytes.

MATERIALS AND METHODS

Hepatocytes and Culture Conditions

The human hepatocytes used in these studies were procured from two sources. Samples HH1–4 were obtained from the Human Cell Culture Center, and samples HH5–7 were acquired from the International Institute for the Advancement of Medicine. Hepatocytes, isolated from whole organ donors using a two-step collagenase procedure [38], were plated in medium supplemented with 5–10% serum for 4–8 hr. Subsequent to isolation of single cell suspensions and prior to arrival at our laboratory, hepatocyte samples were processed as described below.

Hepatocytes isolated from donors HH1 and HH4 were suspended in Euro-Collins solution as described previously [13] and shipped on wet ice. Upon arrival, cell pellets were obtained by centrifugation at 50 *g* for 10 min and plated (Falcon T75 tissue culture flasks) in medium essentially as described [39]. Initial plating density was approximately $1–2 \times 10^7$ cells/flask, which provided $> 85\%$ confluency. Hepatocytes from donors HH2 and HH3 were plated on tissue culture flasks in Williams' E medium immediately following isolation and shipped approximately 24 hr later at ambient temperature. Culture conditions during the initial 24-hr time period were essentially as described by Strom and co-workers [40].

Hepatocytes isolated from samples HH5–7 were plated to near confluency ($\sim 2 \times 10^7$ cells/flask) in Waymouth's Minimal Essential Medium on Primaria plastic (Nunc). After cell attachment, a chemically defined medium was applied [41], and flasks were shipped on wet ice.

Following arrival at our laboratory, flasks were vented and incubated at 37° in 5% CO_2 for approximately 30 min. Adherent cells (generally $> 90\%$ confluency) were washed gently with serum-free Williams' E medium, followed by replacement with a chemically defined Williams' E formulation [39]. Approximately 4 hr later, Matrigel™ (Collaborative Biomedical Products), diluted to 233 μg/mL, was added to the medium and swirled gently into solution [39]. Medium was subsequently changed every 24 hr. Culture conditions similar to those employed in the present study have been shown to result in optimal induction responses in rat primary hepatocytes [10].

Chemical Treatment

Approximately 24 hr following Matrigel™ addition, cells were exposed to prototypic chemical inducers for 24 hr (HH1, 2, 3, 4, and 7) or 48 hr (HH5 and 6). Thus, prior to any chemical treatment, hepatocytes adapted to the new culture conditions for approximately 1 day. The chemicals tested and the concentrations used were as follows: PB (0.5 mM); DEX (1.0 μM); β-NF (22 μM); BHA (25 μM); CIPRO (0.1 mM); and ARO (2000 μg/mL). The DEX concentration in experiments HH5, 6, and 7 was increased to 20 μM. The ARO concentration was decreased to 2 μg/mL in HH5, and to 30 μg/mL in HH6 and HH7. All chemicals were of the highest quality commercially available.

Following chemical treatment, cells were washed briefly with sterile PBS, and collected by scraping with a rubber policeman. Cell pellets, obtained by centrifugation at 50 *g*, were resuspended in a nominal volume of PBS. For protein analysis, a small volume of the cell suspension (~20%) was snap frozen in liquid nitrogen and stored at −80° until further processing.

RNA Analyses

RNA was isolated [42] and processed as described [34]. Cellular RNA expression levels were determined using 5 μg of total RNA applied to a Minifold II slot blot manifold (Schleicher & Schuell), as reported previously [34]. Using oligonucleotide probes, mEH RNA expression was normalized to 18S rRNA levels, after being quantified densitometrically as described [34].

CYP3A mRNA levels were assessed in hybridization assays using a combination of two oligonucleotides that were reported previously [43]. The HP oligomer is complementary to CYP3A3 and 3A4 transcripts, whereas the RP oligomer also hybridizes to CYP3A7 mRNA. To reflect this specificity, mRNA species detected with these probes are collectively referred to as CYP3A.

CYP1A2 mRNA expression was assessed by hybridization to the following probes:

TABLE 1. Hepatocyte donor information

Liver sample	Age (years)	Sex	Race	Cause of death	Drug history*
HH1	19	Male	Caucasian	Gunshot wound	Smoker (2 years)
HH2	72	Male	Caucasian	Cerebral vascular accident	Coumadin, atenolol, digoxin, captopril
HH3	63	Female	Caucasian	Intracerebral bleeding secondary to motor vehicle accident	Coumadin
HH4	5	Female	Black	Head trauma	None
HH5	37	Female	Caucasian	Gunshot wound	Prozac, smoker (20 years)
HH6	59	Male	Caucasian	Cerebral vascular accident	Smoker
HH7	1.67	Male	Hispanic	Head trauma	None

*Drug history refers to pre-hospitalization.

CYP1A2 HP, 5′-

AAC/AAG/GGC/TGA/GTC/CCC/GT-3′

CYP1A2 RP, 5′-

GAG/GTT/GCC/GCT/GGC/TCT/AG-3′

These oligomers do not have significant sequence similarity to other reported CYP genes, especially *CYP1A1* (≤ 14/20 matches). The specificity of oligomer probes was confirmed by northern blot analyses, where the appropriate molecular size for each mRNA species was observed (data not shown).

Immunoreactive mEH Protein Analysis

S-9 protein was isolated as previously described [34]. Ten micrograms of S-9 protein from each sample was size-fractionated in a 10% polyacrylamide gel, transferred to an Immobilon-P membrane (Millipore Corp.), and reacted with mEH antibody [34].

Quantitative Densitometry and Data Analysis

Autoradiograms obtained from slot-blotted RNA, and western-blotted proteins were exposed within the linear range of the X-ray film. Images were quantified as described previously [34], and results are presented either as relative densitometric units, or as normalized values derived from chemically treated cells, relative to control levels. Histograms were generated using Origin software, version 4.10 (Microcal).

RESULTS

Hepatocyte donor information is presented in Table 1. The seven donors include three ethnic groups, both genders, and a broad range of ages (less than 2 years old to 72 years old). In each case, head injury was the primary cause of death. The livers from which hepatocytes were isolated had no reported pathology. Livers were harvested initially with the intent to transplant, but could not be utilized for this purpose.

Cells shipped to our laboratory overnight in suspension (HH1 and HH4) were evaluated for viability by trypan blue exclusion, before plating. Viability was estimated to be > 90%, and the majority of cells adhered to tissue culture flasks within 4 hr of plating. Under phase contrast light microscopy, hepatocytes from each of the seven preparations were morphologically cuboidal and epithelial in appearance. Cell density was sufficiently high in all experiments to permit many areas of cell–cell contact and apparent junction formation. During the experimental duration, there was no obvious indication of cellular toxicity following any chemical treatment, although the appearance of granules within cells was noted in some instances.

Due to the limiting number of hepatocytes available for these analyses, coupled with the objective to evaluate as many potential inducers as possible, it was not feasible to conduct detailed concentration–response optimization experiments. Instead, generally saturating levels of each chemical were selected, based on previous reports of xenobiotic gene modulatory effects observed for PB [11], DEX [9, 44], β-NF [11], ARO [43], CIPRO [45], or BHA (Raaka S and Omiecinski CJ, unpublished observations) in cell culture experiments. Among the seven experiments, however, ARO and DEX were applied at more than one concentration, which permitted effects of concentration to be considered for these chemicals.

The quality of RNA isolated from control and chemically treated hepatocytes was evaluated on 1.25% agarose/2.2 M formaldehyde denaturing gels. Based on the presence of intact 18S and 28S rRNA, degradation was judged to be minimal (data not shown). To maximize quantitation efficiency, RNA expression levels in control and treated cells were determined from five μg of total RNA applied to slot blots and hybridized to gene-specific oligomer probes.

A representative slot blot of mEH and 18S rRNA gene-specific expression, obtained from HH6, is presented in Fig. 1, panel A. RNA expression for mEH was apparent in both uninduced and chemically treated cells, but autoradiographic signal intensity did not vary greatly between treatments. Normalization of mEH RNA expression to the corresponding 18S rRNA level (right column) revealed

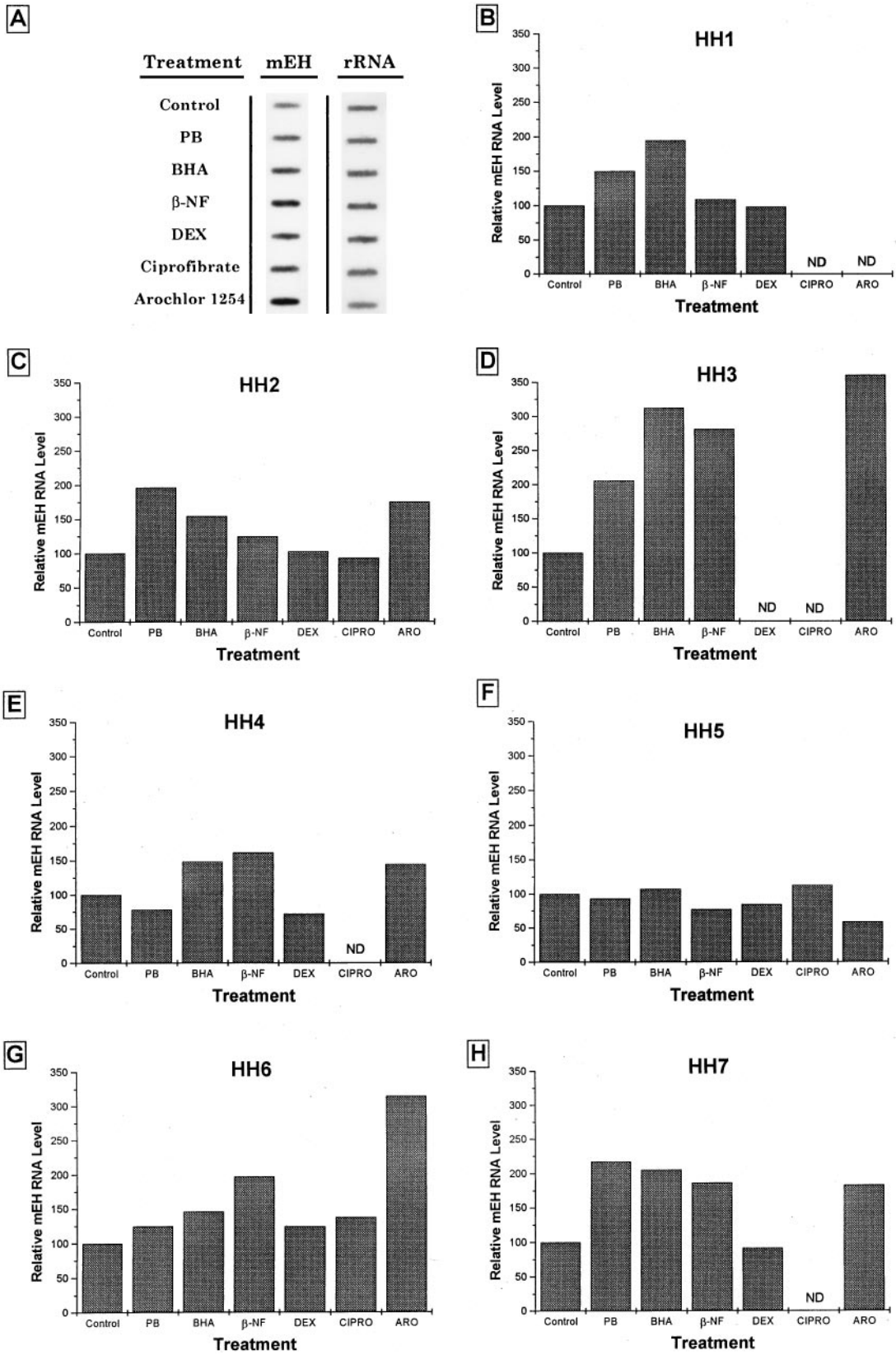

FIG. 1. Primary human hepatocyte expression of mEH RNA. Total RNA from control and chemically treated cells was applied to slot blots, probed with gene-specific oligomers, and quantified densitometrically. Panel A: Representative autoradiograms for mEH RNA and 18S rRNA expression obtained from experiment HH6. Panels B–H: mEH RNA expression after chemical treatments, relative to corresponding untreated cells, for each hepatocyte experiment. mEH RNA expression levels were normalized to 18S rRNA levels for each treatment. ND, not determined.

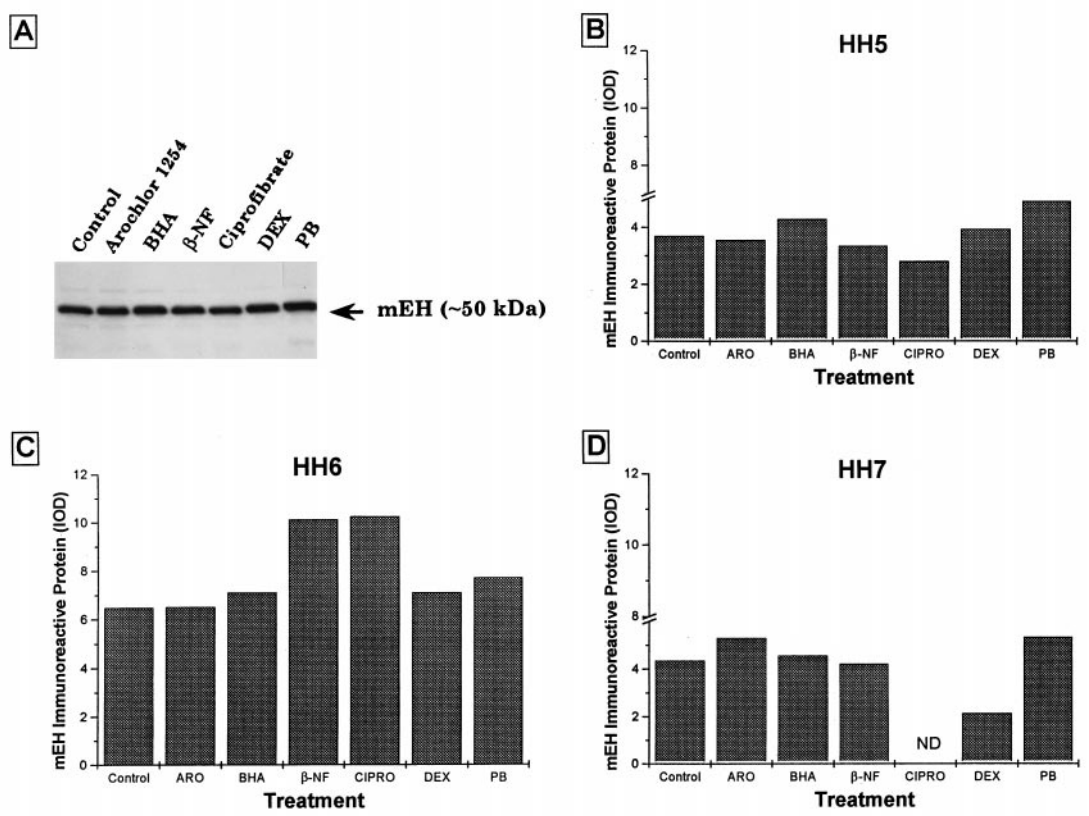

FIG. 2. Expression of mEH protein in primary human hepatocytes. S-9 protein from control and treated cells was size-fractionated, transferred to membranes, and visualized with mEH anti-peptide antibody. Panel A: Representative western immunoblot of mEH protein expression obtained from experiment HH5 demonstrating specificity of mEH antibody. Panels B–D: mEH protein expression in experiments HH5–HH7. Expression levels, measured as integrated optical density (IOD) units, were standardized at 12 for all experiments. ND, not determined.

that cells treated with β-NF and ARO expressed the highest levels of mEH RNA, which were approximately 2- and 3-fold greater, respectively, than those observed in the corresponding untreated cells. Normalization of mEH RNA expression levels relative to the corresponding control values for each of the seven experiments is graphically presented in Fig. 1, panels B–H. In each graph, the ordinate was standardized at 350 (3.5 × control value) to facilitate inter-experimental comparison. As apparent from the figure, effects on mEH steady-state RNA expression were variable for each of the chemical treatments among the different donor hepatocytes. In most cultures, exposure of cells to β-NF and ARO resulted in the greatest accumulation of mEH RNA. In the case of HH5, no increase in mEH RNA level was observed following exposure to either β-NF or ARO; the lack of an ARO-induced response may be due to the lower concentration of chemical applied in this experiment (2 μg/mL).

mEH RNA expression levels following exposure to the other chemicals tested was typically more variable than observed with β-NF and ARO treatment. For instance, PB exposure resulted in 1.5- to 2-fold increases in steady-state mEH RNA expression in experiments HH1, 2, 3, and 7, but had little or no effect on hepatocytes isolated from the other donors. Treatment with BHA also resulted in a highly variable response with 2- to 3-fold increases noted in HH1, 3, and 7 but less effect seen in the other samples. In contrast, there were very modest changes observed in mEH RNA expression following exposure to either DEX or CIPRO.

Cellular S-9 protein was prepared from each of the treatment groups in experiments HH5, 6, and 7, permitting assessment of mEH immunoreactive protein content. Western blotted protein obtained from HH5 (Fig. 2, panel A), characteristic of other experiments, demonstrates the specificity of the anti-mEH antibody. The absence of notable mEH protein variation following chemical exposure was reminiscent of mEH RNA expression observed in HH5 (Fig. 1, panel F). Figure 2, panels B–D, graphically presents mEH immunoreactive protein levels determined in experiments HH5–7. In all cases, mEH protein expression varied moderately in chemically treated cells, relative to controls (< 2-fold maximal change), and no uniform relationship between mEH protein content and steady-state RNA level was apparent. For example, in HH6, ARO treatment did not alter mEH protein content, whereas exposure to this agent resulted in a > 3-fold increase in steady-state mEH RNA. Also in HH6, exposure to CIPRO produced a modest accumulation in mEH RNA (approximately 25% greater than untreated cells), whereas immunoreactive protein

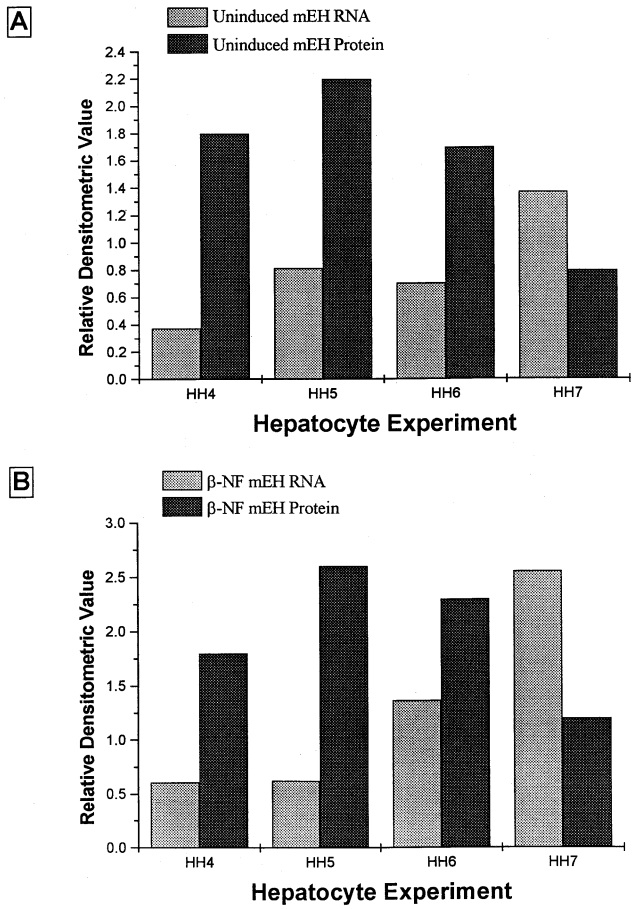

FIG. 3. Comparison of interindividual variation in mEH RNA and protein expression. Panel A: RNA and S-9 protein were isolated from untreated hepatocytes in experiments HH4–HH7, and mEH expression was measured by reactivity with gene-specific oligomer probes and antibody, respectively. Panel B: Relative expression of human mEH RNA and protein in β-NF-treated primary hepatocytes.

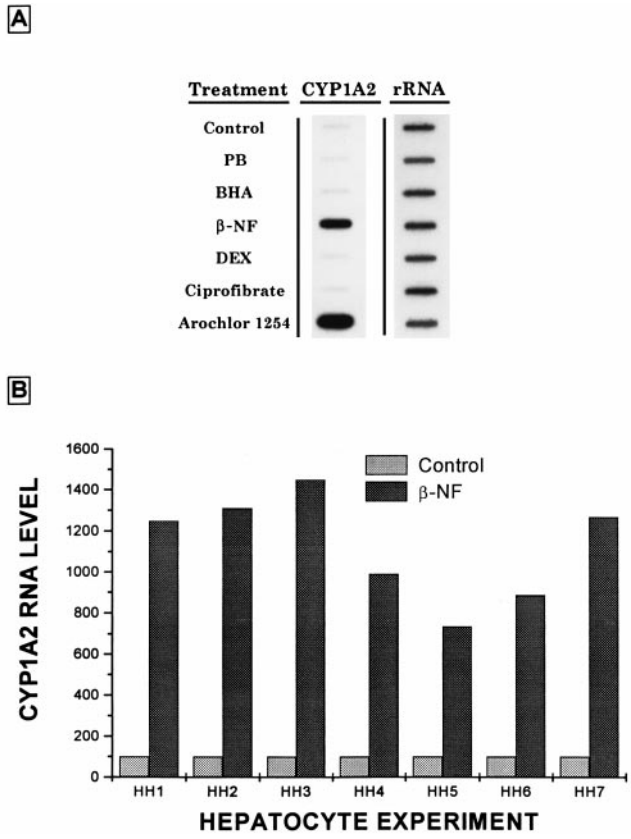

FIG. 4. CYP1A2 mRNA expression in primary human hepatocytes. Panel A: Representative slot blots of CYP1A2 and 18S rRNA expression obtained from HH2. Panel B: Steady-state CYP1A2 RNA expression detected in untreated and β-NF-exposed cells in experiments HH1–HH7. Control levels were arbitrarily assigned a value of 100 to permit inter-experimental comparison.

content was increased to the maximal amount observed in this experiment.

The relationship between mEH RNA and protein expression was determined more explicitly in experiments HH4–7. RNA and S-9 protein isolated from control and β-NF-treated cells were prepared in parallel and directly compared. Figure 3, panel A, depicts a histogram of densitometrically scanned control RNA and protein blots obtained from autoradiographic signals. Although uninduced RNA and protein levels showed a disproportionate positive correlation in HH4, 5, and 6, they were inversely related in HH7. The mEH RNA/protein relationship obtained following treatment with β-NF is presented in panel B. Relative mEH RNA expression was less predictive of immunoreactive protein content compared with the uninduced state. Similar to the untreated hepatocyte preparation, donor HH7 mEH RNA levels were inversely associated with mEH protein following β-NF exposure.

To confirm that the hepatocyte culture conditions were permissive for xenobiotic-metabolizing gene responsiveness following chemical treatment, RNA was isolated from each experiment and analyzed with hybridization probes complementary to two human CYP genes that are regulated by prototypic inducers. The human *CYP1A2* gene is responsive to PAH compounds [46–48], and exposure to PB increases CYP3A mRNA expression [44].

Figure 4, panel A, presents an RNA slot blot derived from HH2 hepatocytes and hybridized to CYP1A2 (left column) or 18S rRNA (right column) probes. Striking increases in levels of CYP1A2 mRNA expression resulted from β-NF or ARO treatment, whereas exposure to the other chemicals tested resulted in minimal change relative to the uninduced state. The CYP1A2 responsiveness to β-NF in each of the seven hepatocyte preparations is shown graphically in Fig. 4, panel B. The expression levels observed in control cultures was arbitrarily assigned a value of 100 to permit interindividual comparison. Hepatocytes from each of the seven experiments displayed pronounced, but varied, β-NF responsiveness. Donor HH3 demonstrated 14-fold induction of CYP1A2 steady-state RNA following β-NF treatment, whereas HH5 exhibited an ~7-fold increase above corresponding control cells.

Figure 5, panel A, presents a slot blot of RNA isolated

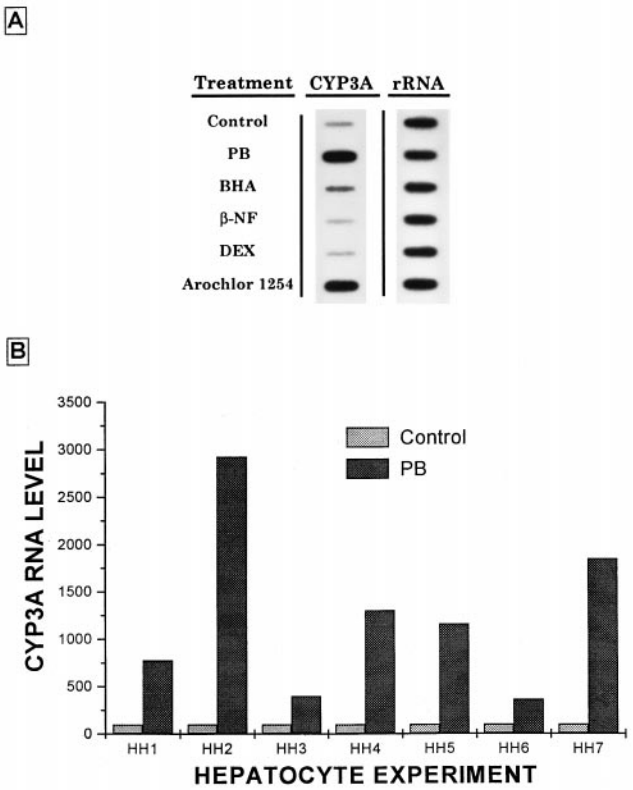

FIG. 5. CYP3A RNA expression in primary human hepatocytes. Panel A: Representative slot blot analyses of CYP3A and 18S rRNA expression obtained from HH7. Panel B: Steady-state CYP3A expression levels in untreated and PB-exposed hepatocytes in experiments HH1–HH7. Control levels were assigned the value of 100 to permit inter-experimental comparison.

from HH7 that was probed with oligomers complementary to CYP3A (left column) or 18S rRNA (right column) genes. Cells treated with PB, ARO, and, to a lesser extent, BHA displayed increased CYP3A RNA expression relative to control cells. Figure 5, panel B, depicts a histogram of the densitometrically scanned levels of CYP3A mRNA isolated from control and PB-treated cells in the seven hepatocyte experiments. Again, control values for each experiment were fixed at 100% to permit comparison. Exposure to PB resulted in remarkably varied CYP3A RNA expression compared with uninduced cells, ranging from approximately 4- to 30-fold (HH6 and HH2, respectively).

Relative variations in mEH, CYP1A2, and CYP3A RNA expression profiles following chemical treatment are, in part, a function of the constitutive expression levels for each of these genes. The interindividual constitutive expression patterns for the respective genes in the different hepatocyte preparations were compared by applying 3 μg of total RNA isolated from each experiment to a single slot-blot membrane that was hybridized to the gene-specific probes, and normalized to 18S rRNA levels. The results of this analysis are summarized as a histogram in Fig. 6. Constitutive RNA expression levels varied approximately 3.9- and 2.6-fold for mEH and CYP1A2, respectively. A remarkable 9.6-fold variation was observed for uninduced CYP3A expression in the seven donor hepatocyte samples.

DISCUSSION

In the current investigation, we used cultures of primary hepatocytes, isolated from non-diseased donor livers, to determine human mEH gene expression patterns following exposure to prototypic chemical inducers. Human hepatocytes maintained in a cell-differentiated state permit the examination of chemically mediated perturbations in gene expression, experiments that cannot be conducted ethically *in vivo*. To our knowledge, this report is the first characterization of the human mEH gene modulatory response in primary hepatocytes following well-defined chemical exposure conditions.

Based on our results obtained from seven different cultures of primary hepatocytes, together with previous analyses of liver samples [34], we conclude that human hepatic mEH RNA is expressed at readily detectable basal levels. Additionally, the results reported here indicate that treatment of primary hepatocytes with prototypic chemical agents does not markedly perturb mEH RNA steady-state expression. For example, exposure to ARO resulted in a maximal 3.5-fold increase in mEH RNA above untreated levels in HH3, with more modest effects in the remaining cultures. It is noteworthy that apparent idiosyncratic changes in mEH RNA expression also were detected following other chemical treatments. PB, BHA, or β-NF treatment in the different hepatocyte cultures resulted in various steady-state mEH RNA expression level changes that were at times increased, decreased, or unchanged relative to the respective constitutive values. These observations support the concept that the mEH steady-state RNA expression levels observed among the samples subsequent to chemical treatments reflects variation in interindividual response capacity. Induction polymorphisms have been postulated for other xenobiotic-metabolizing genes, such as the CYP1A family, following exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) in primary human hepatocytes [49].

Because hepatocyte mEH RNA expression levels were not strikingly altered by chemical exposure, it was necessary to demonstrate that the cells maintained the capacity to respond to xenobiotic treatments. CYP1A2 and CYP3A mRNA expression levels, previously shown to increase following PAH [46–48] and PB [44] treatment, clearly displayed striking chemical responsiveness in each of the seven hepatocyte cultures. These observations indicate that the hepatocytes were in an inducer-functional, if not optimal, state and were capable of genetic response to chemical exposures. Of potential biologic importance, chemical responsiveness of the CYP genes analyzed in this study was not predictive of mEH gene response. For example, β-NF exposure resulted in markedly increased CYP1A2 mRNA levels, but this treatment caused only modest changes in mEH expression.

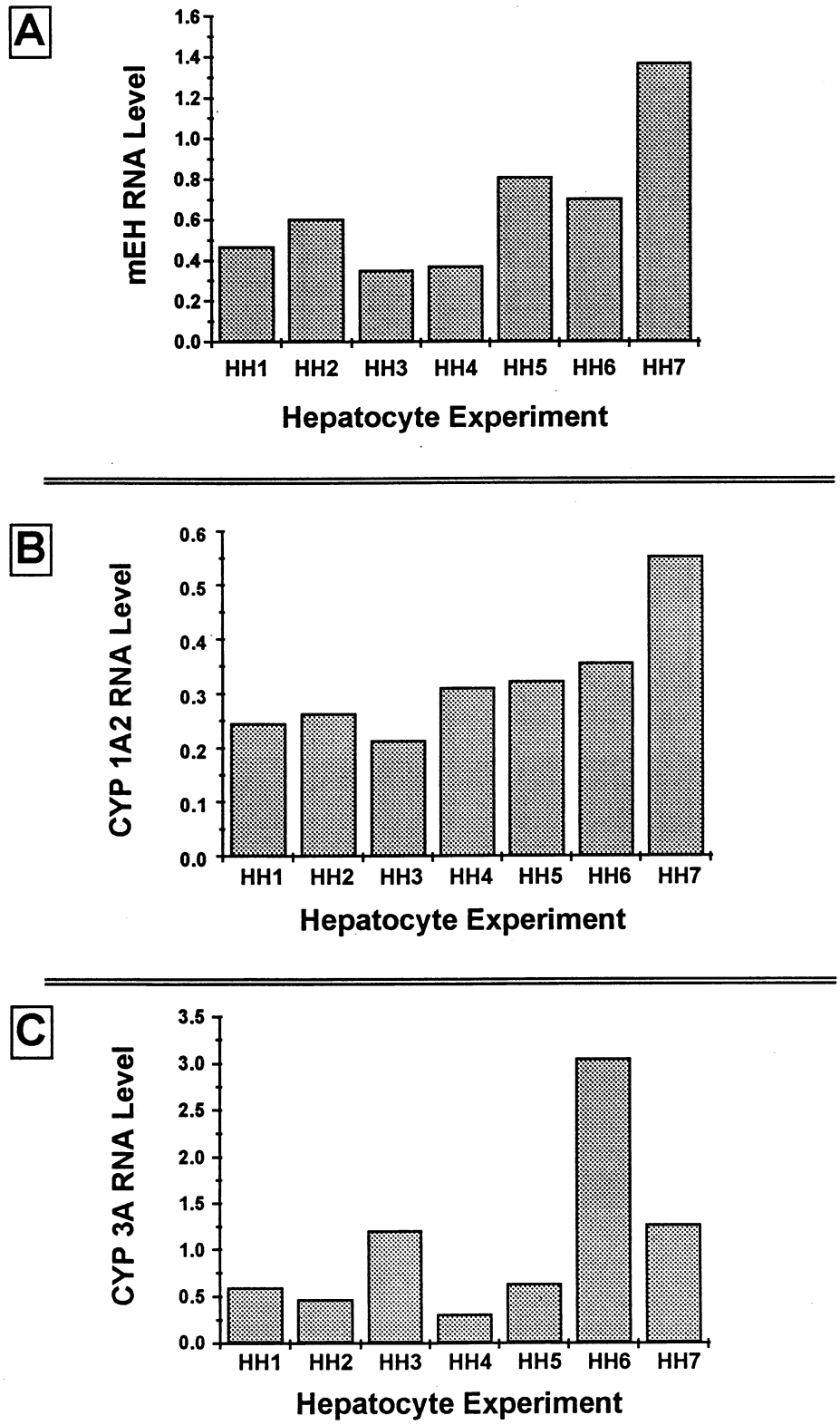

FIG. 6. Variation in constitutive expression of mEH, CYP1A2, and CYP3A RNA in primary human hepatocytes. RNA obtained from untreated cells in the seven experiments was applied to a single membrane and probed with gene-specific oligomers, followed by normalization to 18S rRNA expression.

Constitutive levels of gene expression are important to consider when evaluating chemically modulated mRNA profiles. Because the hepatocytes were in culture for at least 3 days post donor harvest, it is not likely that basal RNA expression estimates were influenced by chemical exposures prior to organ procurement. Donor 6 exemplifies the influence of constitutive expression on gene response appraisal following chemical treatment. These hepatocytes demonstrated the highest basal expression of CYP3A RNA and the lowest relative response to PB among the seven samples evaluated. Characterization of PB responsiveness based solely on CYP3A expression levels relative to control would lead to the potentially erroneous conclusion that these hepatocytes were minimally responsive, when, in fact, the absolute level of PB-induced P4503A mRNA could be similar to other samples.

Assessment of immunoreactive mEH protein content in all treatment groups for three of the seven hepatocyte experiments showed that this parameter generally varied less than the corresponding mRNA value following chemical treatment (at the time points examined). Furthermore, the mEH protein content was poorly correlated with mRNA level. Because mEH RNA and protein expression were assayed at a single time point, it is possible that molecular half-life considerations play an underlying role in this conclusion. However, our laboratory has previously reported a discordant relationship between human mEH RNA and protein levels in multiple contexts [29, 34, 37]. The present work extends these observations to mEH expression profiles in hepatic cells following chemical exposures.

The effects of chemical inducers on mEH expression in human hepatocytes can be compared with results in animal experiments. *In vivo* studies measuring mEH enzymatic activity in mice and rats exposed to PB, BHA, β-NF, or PAH demonstrated changes relative to control values, that were modest and did not exceed 6.5-fold [50, 51]. These results are generally similar to those reported here using primary human hepatocytes. In contrast, *in vivo* exposure to imidazole compounds was reported to transcriptionally activate the rat mEH gene, increasing mRNA levels approximately 10-fold following treatment with ketoconazole or miconazole [52]. We could not reproduce this effect on mEH RNA expression in HH3 following treatment with either 20 μM of ketoconazole or 50 μM of miconazole (data not shown). In fact, mEH levels decreased relative to untreated cells following imidazole treatment (24-hr exposure). Possible reasons for this discrepancy in mEH RNA expression include species differences in induction potential, treatment effects (time from exposure to measurement of RNA expression, or dose), and/or an idiosyncratic response of this hepatocyte donor. Of relevance to these considerations, dramatic intra-species differences have been reported with respect to CYP3A induction in primary hepatocytes [44].

The molecular mechanisms underlying the variation in mEH RNA and protein expression following chemical treatment currently are unknown. It is possible that polymorphic *cis*-acting elements located in regulatory regions of the mEH gene differentially control mRNA levels resulting from exposure to certain inducing agents, and we are currently testing this hypothesis (Raaka S and Omiecinski CJ, unpublished observations). Regulatory mechanisms involving multigenic controls may also be possible. For instance, using differential display and rat embryo fibroblast cells, it has been shown recently that functional p53 protein up-regulates mEH gene expression [53]. Post-transcriptional regulation of mEH expression may constitute another important control mode.

In conclusion, our analyses suggest that exposure of primary human hepatocytes to structurally dissimilar chemical compounds, such as PB and β-NF, results in marked increases in phase I mRNAs (CYP3A and 1A2, respectively), but causes modest alterations in mEH expression. Based on studies conducted with experimental animals, most of the chemicals examined in the present study would be considered bifunctional inducers, capable of modifying both phase I and phase II metabolism. Chemical exposures that result in augmented CYP gene expression likely lead to the increased generation of reactive intermediates by these phase I enzymes. A corresponding modest increase in human mEH and/or glutathione *S*-transferase expression could result in ineffectual detoxification in those instances when phase II activities are rate-limiting. The absence of a coordinated modulation in activation/detoxification pathways subsequent to chemical exposure implies increased risk for genotoxic outcomes, such as teratogenesis and cancer. Future research on the coordinated regulation of these pathways in human cells and tissues is required to enable more accurate predictions of toxic events due to the interplay of induction capacity and chemical exposures.

We thank Brent Bardsley (Human Cell Culture Center), Dr. Stephen Strom (University of Pittsburgh), Claire Lauber (International Institute for the Advancement of Medicine), and Denis Guenette (University of Colorado) for procurement and isolation of hepatocytes. The skillful technical assistance of Lin Jing is gratefully acknowledged. This research was supported by the following grants from the NIH: ES04978, GM32281 (C.J.O.), and ES07032 (E.M.L.). C.J.O. is a Burroughs Welcome Fund Toxicology Scholar.

References

1. Gonzalez FJ, The molecular biology of cytochrome P450s. *Pharmacol Rev* **40:** 243–288, 1989.
2. Paine AJ, Heterogeneity of cytochrome P450 and its toxicological significance. *Hum Exp Toxicol* **14:** 1–7, 1995.
3. Guengerich FP, Characterization of human microsomal cytochrome P-450 enzymes. *Annu Rev Pharmacol Toxicol* **29:** 241–264, 1989.
4. Boobis AR, Sesardic D, Murray BP, Edwards RJ, Singleton AM, Rich KJ, Murray S, de la Torre R, Segura J, Pelkonen O, Pasanen M, Kobauashi S, Zhi-Guang T and Davies DS, Species variation in the response of the cytochrome P-450-dependent monooxygenase system to inducers and inhibitors. *Xenobiotica* **20:** 1139–1161, 1990.
5. Meyer UA, Molecular genetics and the future of pharmacogenetics. *Pharmacol Ther* **46:** 349–355, 1990.

6. Daly AK, Cholerton S, Gregory W and Idle JR, Metabolic polymorphisms. *Pharmacol Ther* **57:** 129–160, 1993.
7. Langouet S, Morel F, Meyer DJ, Fardel O, Corcos L, Ketterer B and Guillouzo A, A comparison of the effect of inducers on the expression of glutathione-*S*-transferases in the liver of the intact rat and in hepatocytes in primary culture. *Hepatology* **23:** 881–887, 1996.
8. Schuetz EG, Li D, Omiecinski CJ, Muller EU, Kleinman HK, Elswick B and Guzelian PS, Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. *J Cell Physiol* **134:** 309–323, 1988.
9. Sidhu JS, Farin FM and Omiecinski CJ, Influence of extracellular matrix overlay on phenobarbital-mediated induction of CYP2B1, 2B2, and 3A1 genes in primary adult rat hepatocyte cultures. *Arch Biochem Biophys* **301:** 103–113, 1993.
10. Sidhu JS, Farin FM, Kavanagh TJ and Omiecinski CJ, Effect of tissue-culture substratum and extracellular matrix overlay on liver-selective and xenobiotic inducible gene expression in primary rat hepatocytes. *In Vitro Toxicol* **7:** 225–242, 1994.
11. Sidhu JS and Omiecinski CJ, Modulation of xenobiotic-inducible cytochrome P450 gene expression by dexamethasone in primary rat hepatocytes. *Pharmacogenetics* **5:** 24–36, 1995.
12. Schuetz EG, Schuetz JD, Thompson MT, Fisher RA, Madariage JR and Strom SC, Phenotypic variability in induction of P-glycoprotein mRNA by aromatic hydrocarbons in primary human hepatocytes. *Mol Carcinog* **12:** 61–65, 1995.
13. Schuetz EG, Schuetz JD, Strom SC, Thompson MT, Fisher RA, Molowa DT, Li D and Guzelian PS, Regulation of human liver cytochromes P-450 in family 3A in primary and continuous culture of human hepatocytes. *Hepatology* **18:** 1254–1262, 1993.
14. Langouët S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich FP, Ketterer B and Guillouzo A, Inhibition of CYP1A2 and CYP3A4 by oltipraz results in reduction of aflatoxin B_1 metabolism in human hepatocytes in primary culture. *Cancer Res* **55:** 5574–5579, 1995.
15. Donato MT, Castell JV and Gómez-Lechón MJ, Effect of model inducers on cytochrome P450 activities of human hepatocytes in primary culture. *Drug Metab Dispos* **23:** 553–558, 1995.
16. Beetham JK, Grant D, Arand M, Garbarino J, Kiyosue T, Pinot F, Oesch F, Belknap WR, Shinozaki K and Hammock BD, Gene evolution of epoxide hydrolases and recommended nomenclature. *DNA Cell Biol* **14:** 61–71, 1995.
17. Hassett C, Turnblom SM, DeAngeles A and Omiecinski CJ, Rabbit microsomal epoxide hydrolase: Isolation and characterization of the xenobiotic metabolizing enzyme cDNA. *Arch Biochem Biophys* **271:** 380–389, 1989.
18. Porter TD, Beck TW and Kasper CB, Complementary DNA and amino acid sequence of rat liver microsomal, xenobiotic epoxide hydrolase. *Arch Biochem Biophys* **248:** 121–129, 1986.
19. Skoda RC, Demierre A, McBride OW, Gonzalez FJ and Meyer UA, Human microsomal xenobiotic epoxide hydrolase. Complementary DNA sequence, complementary DNA-directed expression in COS-1 cells, and chromosomal localization. *J Biol Chem* **263:** 1549–1554, 1988.
20. von Dippe P, Amoui M, Alves C and Levy D, $Na^{(+)}$-dependent bile acid transport by hepatocytes is mediated by a protein similar to microsomal epoxide hydrolase. *Am J Physiol* **264:** G528–G534, 1993.
21. von Dippe P, Amoui M, Stellwagen RH and Levy D, The functional expression of sodium-dependent bile acid transport in Madin-Darby canine kidney cells transfected with the cDNA for microsomal epoxide hydrolase. *J Biol Chem* **271:** 18176–18180, 1996.
22. Alves C, von Dippe P, Amoui M and Levy D, Bile acid transport into hepatocyte smooth endoplasmic reticulum vesicles is mediated by microsomal epoxide hydrolase, a membrane protein exhibiting two distinct topological orientations. *J Biol Chem* **268:** 20148–20155, 1993.
23. Honscha W, Platte HD, Oesch F and Friedberg T, Relationship between the microsomal epoxide hydrolase and the hepatocellular transport of bile acids and xenobiotics. *Biochem J* **311:** 975–979, 1995.
24. Martz F, Failinger C III and Blake DA, Phenytoin teratogenesis: Correlation between embryopathic effect and covalent binding of putative arene oxide metabolite in gestational tissue. *J Pharmacol Exp Ther* **203:** 231–239, 1977.
25. Buehler BA, Delimont D, van Waes M and Finnell RH, Prenatal prediction of risk of the fetal hydantoin syndrome. *N Engl J Med* **322:** 1567–1572, 1990.
26. Friedberg T, Becker R, Oesch F and Glatt H, Studies on the importance of microsomal epoxide hydrolase in the detoxification of arene oxides using the heterologous expression of the enzyme in mammalian cells. *Carcinogenesis* **15:** 171–175, 1994.
27. Gautier JC, Urban P, Beaune P and Pompon D, Simulation of human benzo[*a*]pyrene metabolism deduced from the analysis of individual kinetic steps in recombinant yeast. *Chem Res Toxicol* **9:** 418–425, 1996.
28. Shou M, Gonzalez FJ and Gelboin HV, Stereoselective epoxidation and hydration at the K-region of polycyclic aromatic hydrocarbons by cDNA-expressed cytochromes P450 1A1, 1A2, and epoxide hydrolase. *Biochemistry* **35:** 15807–15813, 1996.
29. Hassett C, Aicher L, Sidhu JS and Omiecinski CJ, Human microsomal epoxide hydrolase: Genetic polymorphism and functional expression *in vitro* of amino acid variants. *Hum Mol Genet* **3:** 421–428, 1994.
30. Gaedigk A, Spielberg SP and Grant DM, Characterization of the microsomal epoxide hydrolase gene in patients with anticonvulsant adverse drug reactions. *Pharmacogenetics* **4:** 142–153, 1994.
31. McGlynn KA, Rosvold EA, Lustbader ED, Hu Y, Clapper ML, Zhou T, Wild CP, Xia X-L, Baffoe-Bonnie A, Ofori-Adjei D, Chen G-C, London WT, Shen F-M and Buetow KH, Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B_1. *Proc Natl Acad Sci USA* **92:** 2384–2387, 1995.
32. Lancaster JM, Brownlee HA, Bell DA, Futreal PA, Marks JR, Berchuck A, Wiseman RW and Taylor JA, Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol Carcinog* **17:** 160–162, 1996.
33. Hassett C, Robinson KB, Beck NB and Omiecinski CJ, The human microsomal epoxide hydrolase gene (EPHX1): Complete nucleotide sequence and structural characterization. *Genomics* **23:** 433–442, 1994.
34. Hassett C, Lin J, Carty CL, Laurenzana EM and Omiecinski CJ, Human hepatic microsomal epoxide hydrolase: Comparative analysis of polymorphic expression. *Arch Biochem Biophys* **337:** 275–283, 1997.
35. Farin FM and Omiecinski CJ, Regiospecific expression of cytochrome P-450s and microsomal epoxide hydrolase in human brain tissue. *J Toxicol Environ Health* **40:** 317–335, 1993.
36. Omiecinski CJ, Aicher L, Holubkov R and Checkoway H, Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. *Pharmacogenetics* **3:** 150–158, 1993.
37. Omiecinski CJ, Aicher L and Swenson L, Developmental expression of human microsomal epoxide hydrolase. *J Pharmacol Exp Ther* **269:** 417–423, 1994.
38. Seglen PO, Preparation of isolated rat liver cells. *Methods Cell Biol* **13:** 29–83, 1976.

39. Sidhu JS and Omiecinski CJ, Forskolin-mediated induction of CYP3A1 mRNA expression in primary rat hepatocytes is independent of elevated intracellular cyclic AMP. *J Pharmacol Exp Ther* **276:** 238–245, 1996.
40. Kostrubsky VE, Strom SC, Wood SG, Wrighton SA, Sinclair PR and Sinclair JF, Ethanol and isopentanol increase CYP3A and CYP2E in primary cultures of human hepatocytes. *Arch Biochem Biophys* **322:** 516–520, 1995.
41. Ledley FD, Adams RM, Soriano HE, Darlington G, Finegold M, Lanford R, Carey D, Lewis D, Baley PA, Rothenberg S, Kay M, Brandt M, Moen R, Anderson WF, Whitington P, Pokorny W and Woo SLC, Development of a clinical protocol for hepatic gene transfer: Lessons learned in preclinical studies. *Pediatr Res* **33:**(4 Pt 1): 313–320, 1993.
42. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
43. Farin FM, Pohlman TH and Omiecinski CJ, Expression of cytochrome P450s and microsomal epoxide hydrolase in primary cultures of human umbilical vein endothelial cells. *Toxicol Appl Pharmacol* **124:** 1–9, 1994.
44. Kocarek TA, Schuetz EG, Strom SC, Fisher RA and Guzelian PS, Comparative analysis of cytochrome P4503A induction in primary cultures of rat, rabbit, and human hepatocytes. *Drug Metab Dispos* **23:** 415–421, 1995.
45. Zangar RC, Woodcroft KJ, Kocarek TA and Novak RF, Xenobiotic-enhanced expression of cytochrome P450 2E1 and 2B1/2B2 in primary cultured rat hepatocytes. *Drug Metab Dispos* **23:** 681–687, 1995.
46. Daujat M, Peryt B, Lesca P, Fourtanier G, Domergue J and Maurel P, Omeprazole, an inducer of human CYP1A1 and 1A2, is not a ligand for the Ah receptor. *Biochem Biophys Res Commun* **188:** 820–825, 1992.
47. Diaz D, Fabre I, Daujat M, Saint Aubert B, Bories P, Michel H and Maurel P, Omeprazole is an aryl hydrocarbon-like inducer of human hepatic cytochrome P450. *Gastroenterology* **99:** 737–747, 1990.
48. Morel F, Fardel O, Meyer DJ, Langouet S, Gilmore KS, Meunier B, Tu C-PD, Kensler TW, Ketterer B and Guillouzo A, Preferential increase of glutathione *S*-transferase class α transcripts in cultured human hepatocytes by phenobarbital, 3-methylcholanthrene, and dithiolethiones. *Cancer Res* **53:** 231–234, 1993.
49. Schrenk D, Stuven T, Gohl G, Viebahn R and Bock KW, Induction of CYP1A and glutathione *S*-transferase activities by 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin in human hepatocyte cultures. *Carcinogenesis* **16:** 943–946, 1995.
50. Hammock BD and Ota K, Differential induction of cytosolic epoxide hydrolase, microsomal epoxide hydrolase, and glutathione *S*-transferase activities. *Toxicol Appl Pharmacol* **71:** 254–265, 1983.
51. Meijer J and DePierre JW, Hepatic levels of cytosolic, microsomal and 'mitochondrial' epoxide hydrolases and other drug-metabolizing enzymes after treatment of mice with various xenobiotics and endogenous compounds. *Chem Biol Interact* **62:** 249–269, 1987.
52. Kim SG, Transcriptional regulation of rat microsomal epoxide hydrolase gene by imidazole antimycotic agents. *Mol Pharmacol* **42:** 273–279, 1992.
53. Madden SL, Galella EA, Riley D, Bertelsen AH and Beaudry GA, Induction of cell growth regulatory genes by p53. *Cancer Res* **56:** 5384–5390, 1996.