Biochemical Pharmacology, Vol. 54, pp. 1127–1131, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00263-3

Sensitivity of CYP1A1 mRNA Inducibility by Dioxin Is the Same in *Cyp1a2(+/+)* Wild-type and *Cyp1a2(−/−)* Null Mutant Mice

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ABSTRACT. In mammals, the induction of experimental porphyria by halogenated aromatic hydrocarbons (HAHs) seems to be influenced by the levels of hepatic CYP1A2. The pharmacokinetics and relative rates of uptake and storage of HAHs in the liver are correlated with hepatic CYP1A2 concentrations. It is possible that these rates of HAH uptake and storage might affect the expression of other HAH-inducible genes. The differential inducibility of liver CYP1A1 mRNA by dioxin was therefore compared in *Cyp1a2(+/+)* wild-type mice, *Cyp1a2(+/−)* heterozygotes, and *Cyp1a2(−/−)* homozygous null mutants. Using doses of dioxin over eight orders of magnitude (from 10^{-12} to 10^{-4} g/kg), we could detect no differences in the sensitivity of CYP1A1 mRNA inducibility. These data indicate that the complete absence of the microsomal CYP1A2 enzyme has no measurable effect on hepatic expression of the *Cyp1a1*, gene, the only other known member of the mammalian *CYP1A* cytochrome P450 subfamily. BIOCHEM PHARMACOL **54**;10:1127–1131, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. [*Ah*] gene battery; AH receptor; CYP1A1 induction; CYP1A2; knockout mouse line; dioxin pharmacokinetics; δ-aminolevulinic acid synthase; experimental porphyria; halogenated hydrocarbons

Following an outbreak of porphyria cutanea tarda among workers in a factory where 2,4,5-trichlorophenoxyacetic acid was being synthesized, TCDD§ was shown to be an extremely potent inducer of hepatic δ-aminolevulinic acid synthase and was implicated as the likely causative agent [1]. A structure-activity analysis of 15 halogenated dibenzo-*p*-dioxins demonstrated a perfect correspondence between whole-animal toxicity data and the capacity of these dibenzo-*p*-dioxin congeners to induce both δ-aminolevulinic acid synthase and CYP1A1 activity in the liver [2]. These results suggested the involvement of the AHR, known to be involved in the induction of more than a dozen genes (reviewed in refs. [3–6]) including the cytochromes P450 CYP1A1 and -1A2.

Experimental porphyria induced by HAHs in mammals has been suspected to be associated with the induction of CYP1A1 and/or -1A2. The susceptibility to porphyria is greater in C57BL/6 inbred mice having the high affinity AHR than in DBA/2 mice having the low affinity AHR [7, 8]. Subsequently, however, no strict correlation between experimental porphyria and mouse strain differences in AHR affinity was found [9, 10], indicating that additional factors might be important. For example, iron and lipid peroxidation were shown to participate in HAH-induced porphyria [11, 12].

In studies with congenic mouse lines [13], the sustained induction of CYP1A1 and/or -1A2 was believed to play a role in the development of porphyria. Using microsomes from 3-methylcholanthrene-treated rat liver (containing induced CYP1A1 and -1A2) and kidney (containing induced CYP1A1 only), Jacobs and co-workers [14] concluded that the CYP1A2-mediated oxidation of uroporphyrinogen is pivotal in HAH-induced porphyria. Some properties by which HAHs might interact with CYP1A2 and affect the course of porphyria seem to include stabilization of the 1A2 enzyme (presumably by virtue of the HAH being tightly bound to the enzyme) and inhibition of certain catalytic activities of the 1A2 enzyme [15–18].

The effect of sequestration of HAHs in the liver might provide an additional important factor in susceptibility to hepatic porphyria correlated with affinity of the AHR. It was shown that the high levels of constitutive CYP1A2 in liver, and the fourfold to fivefold induced levels of 1A2 following exposure of animals to HAHs, seem to act as a "sink" for these HAHs [19–24]. This had been proposed to

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§ *Abbreviations:* AHR, Ah receptor; HAH(s), halogenated hydrocarbon(s); TCDD or dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. By convention, mouse genes include lowercase letters and italics, and other mammalian genes include all capital letters italicized, whereas the cDNA, mRNA, or enzyme (gene product) of all mammals including mouse are depicted in all capital letters that are not in italics.

Received 3 April 1997; accepted 2 June 1997.

explain why the concentration of dioxin and other HAHs is much higher in the livers of mice having the high affinity AHR than in mice having the low affinity AHR [21, 22], but this does not explain the reports [11, 12] of the lack of a strict correlation between experimental porphyria and AHR affinity.

It is possible that differences in the sequestration of HAHs, as well as differences in CYP1A2 levels, might affect the course of porphyria by altering the expression of other HAH-inducible genes. Will the concentration of sequestered HAHs in the liver be proportional to the level of "freely dissociable" HAH able to interact with the AHR and induce AHR-regulated genes? Such questions can be explored in the viable and fertile *Cyp1a2(−/−)* knockout mouse line recently generated by this laboratory [25].

Compared with the *Cyp1a2(−/−)* mouse, in the *Cyp1a2(+/+)* wild-type mouse, having high basal levels and TCDD-inducible CYP1A2, HAHs such as TCDD are stored at 10- to 50-fold higher concentrations in the liver.* One possibility (see Fig. 1, top) is that if the TCDD is bound to the 1A2 enzyme, this could lead to a lower pool of free TCDD; therefore, less TCDD would be available to interact with the AHR and induce such genes as *Cyp1a1*. *Cyp1a1* gene expression would therefore be low. On the other hand (Fig. 1, bottom), the complete absence of any CYP1A2 protein in *Cyp1a2(−/−)* mice might lead to a greater pool of free TCDD (or other HAH) in the liver, perhaps resulting in more freely dissociable TCDD available for inducing the *Cyp1a1* gene at lower TCDD doses. *Cyp1a1* gene expression would therefore be high. It is also conceivable that the converse might be true, i.e. that the *Cyp1a2(+/+)* mouse might have a larger pool of free TCDD and the *Cyp1a2(−/−)* mouse might have a smaller pool of free TCDD.

Between *Cyp1a2(+/+)* and *Cyp1a2(−/−)* mice, it is thus possible that there would be observable differences in the expression of a dioxin-inducible gene if the CYP1A2 protein serves as an HAH sink. We regard the *Cyp1a1* gene as a very sensitive indictor of dioxin inducibility. The purpose of this report is to compare the differential inducibility of CYP1A1 mRNA by TCDD in *Cyp1a2(+/+)* wild-type mice, *Cyp1a2(+/−)* heterozygotes, and *Cyp1a2(−/−)* homozygous null mutants. We used doses of TCDD over eight orders of magnitude (from 10^{-12} to 10^{-14} g/kg).

MATERIALS AND METHODS

Treatment of Animals

Four to six-week-old *Cyp1a2(+/+)*, *Cyp1a2(+/−)*, and *Cyp1a2(−/−)* mice were used from this laboratory's mouse colony. Generation of the *Cyp1a2(−/−)* knockout mouse line [25] resulted in offspring with mixed genetic background (129/Ola (agouti, having poor affinity AHR) and C57BL/6 (nonagouti, having high-affinity AHR). The problem of the lack of genetic homogeneity in a freshly generated knockout mouse line has been recently discussed [26]. Our *Cyp1a2(−/−)* mice have therefore been bred repeatedly into the C57BL/6 such that the line now contains >95% C57BL/6 genetic background.

We used 10 groups of three each of the *Cyp1a2(+/+)*, *Cyp1a2(+/−)*, and *Cyp1a2(−/−)* genotypes for a total of 90 mice. Intraperitoneal doses of TCDD over eight orders of magnitude were administered (from 1 pg/kg to 100 μg/kg) for 12 hr. The control group received vehicle only (*p*-dioxane, 0.5 ml/kg). All mice were killed at 12 hr, and total liver RNA was prepared. The three livers from each group were pooled for a total of 30 samples.

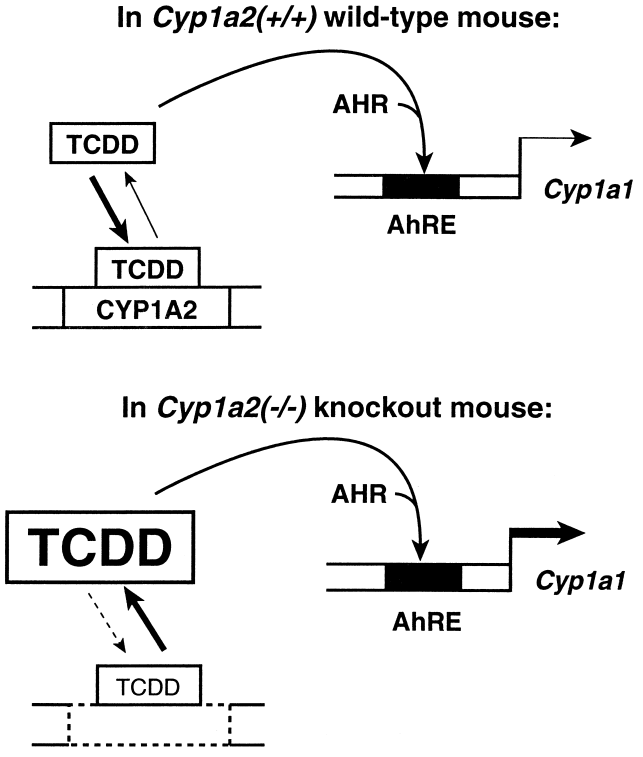

FIG. 1. Illustration of the hypothesis for this study. The wild-type *Cyp1a2(+/+)* mouse has normal high basal and HAH-inducible levels of the hepatic microsomal CYP1A2 enzyme, which binds and sequesters TCDD. The *Cyp1a2(−/−)* knockout mouse has no basal or inducible CYP1A2 enzyme, which might free up more available freely dissociable TCDD (denoted by the larger "TCDD" in rectangle) for the induction of genes such as *Cyp1a1*. The ligand TCDD binds to the AHR and, with the Ah receptor nuclear translocator and probably additional transcription factors, binds to the DNA motif termed the aromatic hydrocarbon response element (AhRE; also called XRE, DRE), leading to up-regulation of the *Cyp1a1* gene. The complete absence of the microsomal CYP1A2 enzyme (shown by dashed rectangle, at bottom) thus might alter dioxin-inducible gene expression, e.g. causing *Cyp1a1* induction to occur at lower concentrations of TCDD (denoted by the larger arrow over *Cyp1a1*, at bottom).

Northern Hybridization Analysis

Total RNA was isolated by the acid guanidinium isothiocyanate extraction method [27]. The RNA (30 μg) was

* Linda S. Birnbaum, personal communication.

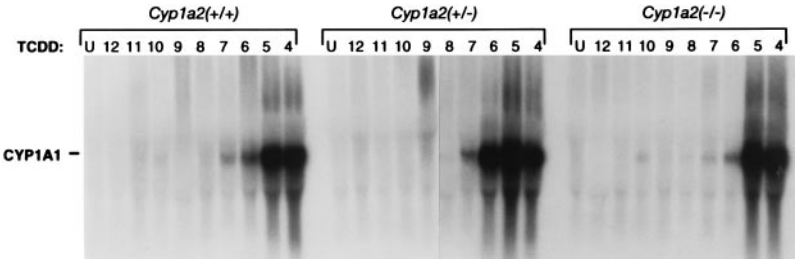

FIG. 2. Northern hybridization analysis of hepatic CYP1A1 mRNA from the three genotypes: the *Cyp1a2(+/+)* wild-type, the *Cyp1a2(+/−)* heterozyate, and the *Cyp1a2(−/−)* homozygous null mouse. TCDD treatment was administered over eight orders of magnitude (from 10^{-12} to 10^{-4} g/kg), and the leftmost lane of each set of ten represents the untreated control (U).

loaded onto 1% agarose-formaldehyde gels, transferred to nylon membranes (Nytran Plus, Schleicher and Schuell), and UV-crosslinked. Prehybridization and hybridization were performed in 10% dextran sulfate, 1% SDS, and 6 × SSC. Membranes were probed with the 1.2-kb 3′-specific fragment of the mouse CYP1A1 cDNA [28]. The densities of the 18 S and 28 S rRNA bands on ethidium bromide-stained gels were used as an RNA-loading control. Hybridization was performed at 60°, and the blots were washed at 60° prior to autoradiography. The blots were then semi-quantitated by scanning densitometry of the CYP1A1 mRNA to [18 S + 28 S] rRNA ratio [29]. The hybridization analysis of the 30 RNA samples was repeated two additional times. Statistical analysis of the data was performed by Student's two-tailed *t* test.

RESULTS AND DISCUSSION

No effect on CYP1A1 mRNA inducibility

Northern blot analysis of hepatic CYP1A1 mRNA in control and TCDD-treated mice of all three genotypes is shown in Fig. 2. Following 12 hr of treatment over eight orders of magnitude of TCDD dosage, there were no detectable differences among the three groups. Because the "TCDD sink" effect is maximal in the first several hours following treatment [19–24], we believe that measurements of CYP1A1 mRNA at the 12-hr time point should detect the maximal effect.

A histogram summarizing the experimental data is shown in Fig. 3. Statistically significant ($p < 0.05$) increases in CYP1A1 mRNA, as compared with the untreated controls, were not seen until the doses reached 0.1 μg of TCDD/kg (10^{-7} g/kg) and higher. Furthermore, we found no differences in the sensitivity of CYP1A1 mRNA inducibility by dioxin among the *Cyp1a2(+/+)* wild-type mice, the *Cyp1a2(+/−)* heterozygotes, and the *Cyp1a2(−/−)* homozygous null mutants. We conclude that the complete absence of the CYP1A2 enzyme has no significant effect on hepatic expression of the *Cyp1a1* gene, the only other known member of the mammalian *CYP1A* subfamily.

Comparing our *Cyp1a2(−/−)* knockout with the wild-type *Cyp1a2(+/+)* mouse, Birnbaum and co-workers have determined that dioxin is sequestered 10–50 times less in

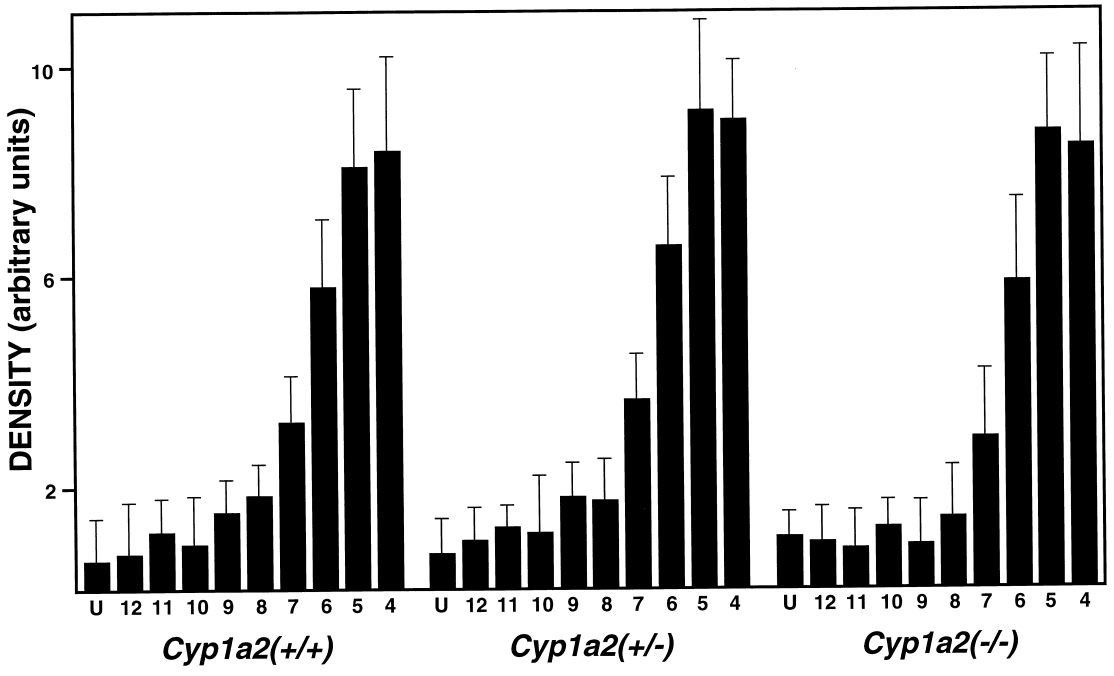

FIG. 3. Histogram of all experimental data, summarized in the same order and labeled the same as in Fig. 2. The arbitrary units represent the semiquantitative densitometry of the CYP1A1 mRNA to [18 S + 28 S] rRNA ratio. Bars and brackets denote means ± S.D. (*n* = three experiments).

the liver of animals having no CYP1A2.* Hence, if the sequestration of TCDD or other HAHs in liver is proportional to the levels of the microsomal CYP1A2 enzyme, there is no evidence that this would cause marked alterations in CYP1A1 mRNA differential inducibility, which we regard as a very sensitive indicator of a TCDD pool "available" for dioxin-inducible gene expression. It is worth noting that the relative affinity of TCDD is about three orders of magnitude higher for the AHR than for CYP1A2 and that the relative content of hepatocyte CYP1A2 is five to six orders of magnitude greater than that of the AHR in the liver cell. The amount of hepatic TCDD sequestration (i.e. the presence or complete absence of CYP1A2 enzyme) thus does not appear to alter the AHR-mediated *Cyp1a1* up-regulation in the liver.

Another possible explanation as to why TCDD sequestration does not alter AHR-mediated up-regulation of *Cyp1a1* has to do with the definition of "reversible" vs. "relatively irreversible" binding of TCDD to CYP1A2. If this binding is reversible, then the pool of "free TCDD" is actually the same (area under the curve) in *Cyp1a2(+/+)* and *Cyp1a2(−/−)* mice, since this would be governed by clearance. The actual shape of the "free TCDD" exposure curve would be altered, however, during longer, more sustained concentrations of free TCDD. On the other hand, if CYP1A2 is involved in the irreversible clearance (e.g. metabolism) of TCDD or other HAH, then the binding becomes "irreversible" to an extent, and lower "free TCDD" exposure will result.

Polymorphism of the Human CYP1A2 Gene

Human differences in hepatic CYP1A2 mRNA levels [30], in hepatic CYP1A2 protein concentrations by Western immunoblot [31], and in urinary metabolites of caffeine [32, 33] suggest that an important *CYP1A2* gene polymorphism likely exists. A trimodal distribution of the enzymatic activity, as determined by urinary caffeine metabolites (consistent with high/high, high/low, and low/low genotypes), has been detected in four separate populations (reviewed in ref. [34]). The genetic basis of this polymorphism has been extensively searched for (inside as well as in the 5′ and 3′ flanking regions of the human *CYP1A2* gene), but, to date, nothing has been found [35].

It is likely this human *CYP1A2* polymorphism might explain differences in risk of certain types of environmentally induced cancer and toxicity. For example, it is possible that, when exposed to identical levels of occupationally hazardous HAHs, workers with genetically high hepatic CYP1A2 levels might have a greater risk of HAH-induced porphyria cutanea tarda than workers with genetically low CYP1A2 levels. The data in this report, however, would suggest that even striking differences in CYP1A2 enzyme levels do not perturb the normal expression of the dioxin-inducible *Cyp1a1* gene. These results are a necessary prelude to studies of experimental porphyria in these mouse lines, which are currently underway.*

We appreciate the help of Robin S. Matlib on scanning densitometry. We thank our colleagues—especially Tim Dalton, Judy Harrer, Alvaro Puga, Jacqueline A. Sinclair, Peter R. Sinclair and Andrew G. Smith—for valuable discussions and a critical reading of this manuscript. Supported in part by NIH Grants R01 ES06321 and 5 P30 ES06096.

References

1. Poland A and Glover E, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: a potent inducer of δ-aminolevulinic acid synthetase. *Science* **179:** 476–477, 1973.
2. Poland A and Glover E, Chlorinated dibenzo-*p*-dioxins: potent inducers of δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase II. A study of the structure-activity relationship. *Mol Pharmacol* **9:** 736–747, 1973.
3. Nebert DW, The *Ah* locus: genetic differences in toxicity, cancer, mutation and birth defects. *Crit Rev Toxicol* **20:** 153–174, 1989.
4. Swanson HI and Bradfield CA, The AH-receptor: genetics, structure and function. *Pharmacogenetics* **3:** 213–230, 1993.
5. Nebert DW, Puga A and Vasiliou V, Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer and in signal transduction. *Ann NY Acad Sci* **685:** 624–640, 1993.
6. Birnbaum LS, The mechanism of dioxin toxicity: relationship to risk assessment. *Environ Health Perspect* **102:** 157–167, 1994.
7. Jones KG and Sweeney GD, Dependence of the porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin upon inheritance of aryl hydrocarbon hydroxylase responsiveness. *Toxicol Appl Pharmacol* **53:** 42–49, 1980.
8. Urquhart AJ, Elder GH, Roberts AG, Lambrecht RW, Sinclair PR, Bement WJ, Gorman N and Sinclair JA, Uroporphyria produced in mice by 20-methylcholanthrene and 5-aminolaevulinic acid. *Biochem J* **253:** 357–362, 1988.
9. Greig JB, Francis JE, Kay SJE, Lovell DP and Smith AG, Incomplete correlation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin hepatotoxicity with Ah phenotype in mice. *Toxicol Appl Pharmacol* **74:** 17–25, 1984.
10. Smith AG and Francis JE, Genetic variation of iron-induced uroporphyria in mice. *Biochem J* **291:** 29–35, 1993.
11. Smith AG and Francis JE, Synergism of iron and hexoachlorobenzene inhibits hepatic uroporphyrinogen decarboxylase in inbred mice. *Biochem J* **241:** 909–913, 1983.
12. Alleman MA, Koster JF, Wilson JHP, Edixhoven-Bosdijk A, Slee RG, Kroos MJ and von Eijk HG, The involvement of iron and lipid peroxidation in the pathogenesis of HCB-induced porphyria. *Biochem Pharmacol* **34:** 161–166, 1985.
13. Hahn ME, Gasiewicz TA, Linko P and Goldstein JA, The role of the Ah locus in hexachlorobenzene-induced porphyria: studies in congenic C57BL/6J mice. *Biochem J* **254:** 245–254, 1988.
14. Jacobs JM, Sinclair PR, Bement WJ, Lambrecht RW, Sinclair JF and Goldstein JA, Oxidation of uroporphyrinogen by methylcholanthrene-induced P-450. *Biochem J* **258:** 247–253, 1989.
15. Voorman R and Aust SD, Inducers of cytochrome P-450d: influence on microsomal catalytic activities and differential

* Linda S. Birnbaum, personal communication.

* Sinclair PR, Dalton T, Sinclair JF, Smith AG and Nebert DW, manuscript in preparation.

regulation by enzyme stabilization. *Arch Biochem Biophys* **262:** 76–84, 1988.
16. Miller DM, Aust AE, Voorman R and Aust SD, Inhibition of 2-aminofluorene mutagenesis in bacteria by inducers of cytochrome P450d. *Carcinogenesis* **9:** 327–329, 1988.
17. Jacobs JM, Sinclair PR, Lambrecht RW, Sinclair JF and Jacobs NJ, Role of inducer binding in cytochrome P-450 IA2-mediated uroporphyrinogen oxidation. *J Biochem Toxicol* **5:** 193–199, 1990.
18. Van Birgelen APJM, Fase KM, van der Kolk J, Poiger H, Brouwer A, Seinen W and van den Berg M, Synergistic effect of 2,2′, 4,4′, 5,5′-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic porphyrin levels in the rat. *Environ Health Perspect* **104:** 550–557, 1996.
19. Kuroki J, Koga N and Yoshimura H, High affinity of 2,3,4,7,8-pentachlorodibenzofuran to cytochrome P-450 in the hepatic microsomes of rats. *Chemosphere* **15:** 731–738, 1986.
20. Voorman R and Aust SD, Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome P450d to the cytochrome and inhibition of its estradiol 2-hydroxylase activity. *Toxicol Appl Pharmacol* **90:** 69–78, 1987.
21. Poland A, Teitelbaum P and Glover E, [^{125}I]2-Iodo-3,7,8-tetrachlorodibenzo-*p*-dioxin-binding species in mouse liver induced by agonists for the Ah receptor: characterization and identification. *Mol Pharmacol* **36:** 113–120, 1989.
22. Poland A, Teitelbaum, P, Glover E and Kende A, Stimulation of in vivo hepatic uptake and in vitro hepatic binding of [^{125}I]2-iodo-3,7,8-tetrachlorodibenzo-*p*-dioxin by the administration of agonist for the Ah receptor. *Mol Pharmacol* **36:** 121–127, 1989.
23. Kedderis LB, Diliberto JJ, Linko P, Goldstein JA and Birnbaum LS, Disposition of 2,3,7,8-tetrabromodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat: biliary excretion and induction of cytochromes CYP1A1 and CYP1A2. *Toxicol Appl Pharmacol* **111:** 163–172, 1991.
24. Kedderis LB, Mills JJ, Andersen ME and Birnbaum LS, A physiologically based pharmacokinetic model for 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in the rat: tissue distribution and CYP1A induction. *Toxicol Appl Pharmacol* **121:** 87–98, 1993.
25. Liang HC, Li H, McKinnon RA, Duffy JJ, Potter SS, Puga A and Nebert DW, *Cyp1a2(−/−)* null mutant mice develop normally, but show deficient drug metabolism. *Proc Natl Acad Sci USA* **93:** 1671–1676, 1996.
26. Nebert DW and Duffy JJ, How knockout mouse lines will be used to study the role of drug-metabolizing enzymes and their receptors during reproduction, development, and environmental toxicity, cancer and oxidative stress. *Biochem Pharmacol* **53:** 249–254, 1997.
27. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
28. Kimura S, Gonzalez FJ and Nebert DW, The murine Ah locus: comparison of the complete cytochrome P_1-450 and P_3-450 cDNA nucleotide and amino acid sequences. *J Biol Chem* **259:** 10705–10713, 1984.
29. Burcham T, Scan analysis: the densitometer for the Macintosh. Biosoft, Ferguson, MO, 1989.
30. Ikeya K, Jaiswal AK, Owens RA, Jones JE, Nebert DW and Kimura S, Human CYP1A2: sequence, gene structure, comparison with the mouse and rat orthologous gene, and differences in liver 1A2 mRNA expression. *Mol Endocrinol* **3:** 1399–1408, 1989.
31. Shimada T, Yun CH, Hiroshi Y, Gautier JC, Beaune PH and Guengerich FP, Characterization of human lung microsomal cytochrome P4501A1 and its role in the oxidation of chemical carcinogens. *Mol Pharmacol* **41:** 856–864, 1992.
32. Butler MA, Lang NP, Young JF, Caporaso NE, Vineis P, Hayes RB, Teitel CH, Massengill JP, Lawsen MF and Kadlubar FF, Determination of CYP1A2 and acetyltransferase phenotype in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics* **2:** 116–127, 1992.
33. Tang BK, Zhou Y and Kalow W, Caffeine as a probe for CYP1A2 activity: potential influence of renal factors on urinary phenotypic trait measurements. *Pharmacogenetics* **4:** 117–124, 1994.
34. Nebert DW, McKinnon RA and Puga A, Human drug-metabolizing enzyme polymorphisms: effects on risk of toxicity and cancer. *DNA Cell Biol* **15:** 273–280, 1996.
35. Yokoi T, Sawada M and Kamataki T, Polymorphic drug metabolism: studies with recombinant Chinese hamster cells and analyses in human populations. *Pharmacogenetics* **5:** S65–S69, 1995.