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Hyper- and Hypo- Induction of Cytochrome P450 activities with Aroclor 1254 and 3-Methylcholanthrene in *Cyp1a2*($-/-$) mice

Melissa L. Barker¹, Laura B. Hathaway¹, Dorinda D. Arch¹, Mark L. Westbroek¹, James P. Kushner², John D. Phillips², and Michael R. Franklin¹

¹ University of Utah Department of Pharmacology and Toxicology, Salt Lake City, UT 84112

² University of Utah Department of Internal Medicine, Salt Lake City, UT 84132

Abstract

The response of hepatic mono-oxygenase activities to Aroclor 1254 or 3-methylcholanthrene was investigated in wild-type and *Cyp1a2*($-/-$) mice. Cytochrome P450 concentrations were similar in naïve *Cyp1a2*($-/-$) and wild-type mice. There was no difference between naïve wild-type and *Cyp1a2*($-/-$) animals in 7-ethoxyresorufin and 7-ethoxy-4-trifluoromethylcoumarin dealkylase activities, nor was the induction response after 3-methylcholanthrene any different between the two genotypes. However, both activities were induced to a higher extent in *Cyp1a2*($-/-$) mice after Aroclor 1254. In contrast, 7-pentoxoresorufin dealkylation activity was lower in *Cyp1a2*($-/-$) mice and this differential was maintained during induction by both agents. 7-Methoxy- and 7-benzoxoresorufin dealkylation activities were also lower than wild-type in naïve *Cyp1a2*($-/-$) animals and during 3-methylcholanthrene induction, but showed accelerated induction in *Cyp1a2*($-/-$) mice with Aroclor 1254. Bufuralol 1'- and testosterone 6 β -hydroxylation activities, and P450 characteristics were evaluated 48 hours after inducer administration. Bufuralol 1'-hydroxylation, a sexual dimorphic activity (female > male) showed no genotype differences in naïve animals. Activity changes varied across gender and genotype, with 3-methylcholanthrene and Aroclor 1254 inducing in male *Cyp1a2*($-/-$), and Aroclor 1254 inducing in female wild-type. Testosterone 6 β -hydroxylation activity was 16% higher in *Cyp1a2*($-/-$) mice and neither 3-methylcholanthrene nor Aroclor 1254 elicited induction. After Aroclor 1254, a 24% increase in P450 concentration with a hypsochromic shift in the ferrous-CO maximum characteristic of CYP1A enzymes occurred in wild-type, compared to no change in either parameter in *Cyp1a2*($-/-$) mice. Induction changes with 3-methylcholanthrene were greater in wild-type mice, a 60% increase in concentration and ~2 nm hypsochromic shift versus a 10% increase and ~1 nm hypsochromic shift in *Cyp1a2*($-/-$) mice. The study demonstrates that deletion of a single P450 can profoundly affect the induction response, as monitored with activities of other P450s, in a manner unrelated to the contribution of the deleted P450 to the activity.

Keywords

Cytochrome P450; Aroclor 1254; Mono-oxygenase activity; *Cyp1a2*($-/-$); 3-Methylcholanthrene

Corresponding author: Michael R. Franklin, University of Utah, Pharmacology and Toxicology, 30 South 2000 East Room 201, Salt Lake City, UT 84112, Phone #: 801-581-7014, Fax#: 801-585-5111, michael.franklin@pharm.utah.edu.

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1. Introduction

CYP1A2 catalyzes the metabolism of drugs and xenobiotics, including many where metabolism results in bioactivation to toxic reactive intermediates. However, CYP1A2 is also involved in the metabolism of endogenous compounds, notably in the hydroxylation of estradiol. *Cyp1a2*($-/-$) null mutant mice show no abnormal phenotype, and are completely viable and fertile [1]. It has been reported that *Cyp1a2*($-/-$) mice exhibit 50% less hepatic fat, a change that appears to correlate with a *Cyp1a2*($-/-$) influence on genes affecting lipogenesis, fatty acid metabolism and cholesterol biosynthesis [2]. When *Cyp1a2*($-/-$) mice are challenged with drugs known to be metabolized by Cyp1a2, they exhibit exaggerated responses and extended half lives [1,3]. Mutant mice also exhibit reduced 4-aminobiphenyl-induced methemoglobinemia [4] and hepatic injury from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure [5]. An absence of Cyp1a2 does not appear to dramatically influence enzyme induction by xenobiotics, the ability of TCDD to induce Cyp1a1 was unaltered [6]. In a study corroborating an absence of mutual influence, constitutive Cyp1a2 expression was not altered by the absence of Cyp1a1 [7].

A significant interest in deletion of Cyp1a2 has arisen from the intimate association of Cyp1a2 with the development of uroporphyrin. TCDD and other aromatic hydrocarbon (Ah) receptor agonists capable of inducing Cyp1a2 have been used to precipitate uroporphyrin in animal models of porphyria cutanea tarda [8,9,10,11,12]. One of our animal models routinely uses Aroclor 1254, a mix of polychlorinated biphenyls (PCBs) with an average 54% chlorine content (by weight) to precipitate uroporphyrin in mice. In contrast to wild-type animals, uroporphyrin does not develop in *Cyp1a2*($-/-$) mice treated with TCDD or PCBs [5,12,13,14]. A partially oxidized uroporphyrinogen has been identified as a competitive inhibitor of uroporphyrinogen decarboxylase. When decarboxylase activity is sufficiently depressed *in vivo*, uroporphyrins accumulate, leading to the observed uroporphyrin. The molecular details of the formation of this partially oxidized porphyrinogen inhibitor molecule have not been determined, however, a role for Cyp1a2 is inferred. While ensuring that the *Cyp1a2*($-/-$) mice treated with Aroclor 1254 were in all other respects identical in oxidizing enzymes possibly involved in this partial oxidation, i.e., cytochrome P450s, we were surprised in a preliminary investigation to find 7-pentoxoresorufin O-depentylation activity (PROD) to be diminished relative to wild-type animals. Investigations with expressed enzymes have characterized PROD as a monitor of Cyp1b1 and Cyp2b20 in mice, not Cyp1a2 [15]. 7-Methoxyresorufin O-demethylation activity (MROD) is a monitor of Cyp1a1, Cyp1a2 and Cyp1b1 and this activity was not deficient relative to wild-type in Aroclor 1254-induced animals, it was in fact considerably higher. We therefore initiated an examination of a range of cytochrome P450 enzyme activities across the initial time course of Aroclor 1254 induction, and the outcome of these investigations is the subject of this report. The data demonstrate that deletion of one cytochrome P450 can profoundly affect the induction response of others. Comparable investigations with the Ah receptor agonist 3-methylcholanthrene (3MC) revealed that an altered induction response was also dependent on the inducing agent utilized.

2. Materials and methods

2.1. Animals

Breeding stock *Cyp1a2*($-/-$) mice on a C57Bl/6J background were a generous gift from Dr. Daniel W. Nebert, University of Cincinnati. These and wild-type animals were generated in house under a University of Utah approved animal protocol. Mice administered 3-methylcholanthrene (3MC) (20 mg/kg) or Aroclor 1254 (133 mg/kg) received the inducers in corn oil vehicle, i.p. All procedures involving animals were approved by the University of Utah Animal Care and Use Committee and were undertaken in concordance with NIH guidelines for the humane care of laboratory animals.

2.2. Hepatic Microsome Preparation

At sacrifice, livers were rapidly excised and the gall bladders removed. Livers were weighed, and homogenized (20% w/v) in 0.25 M sucrose and microsomal suspensions were prepared from the $105,000 \times g$ pellet of the supernatant of successive $10,000 \times g$ and $18,000 \times g$ centrifugations. The protein content of the microsomal suspension in 50 mM Tris-0.25 M sucrose (pH 7.4) was determined using Folin-Ciocalteu reagent [16] and the microsomes stored at -80° until assayed.

2.3. Microsomal Fraction Assays

The cytochrome P450 concentration was quantified from the dithionite reduced ferrous heme-carbon monoxide complex using the extinction coefficient of $91 \text{ mM}^{-1} \text{ cm}^{-1}$ for the 450 (± 1) versus 490 nm wavelength pair. Cytochrome P450 mono-oxygenase activities towards 7-methoxy-, 7-ethoxy- (Sigma-Aldrich®, St. Louis, MO), 7-pentoxy- and 7-benzoxoresorufin (Invitrogen™, Carlsbad, CA) and 7-ethoxy-4-trifluoromethylcoumarin (Sigma-Aldrich®, St. Louis, MO) were determined from the rate of fluorescence increase due to the formation of the resorufin (Ex 544 nm, Em 612 nm) or 7-hydroxy-4-trifluoromethylcoumarin (Ex 409 nm, Em 550 nm) products. Bufuralol (BD Biosciences, San Jose, CA) 1'-hydroxylation and testosterone (Sigma-AldrichR®, St. Louis, MO) 6 β -hydroxylation were determined by HPLC analysis of the microsomal incubation extract. 6 β -Hydroxytestosterone was detected from its absorbance at 236 nm and 1'-hydroxybufuralol by its fluorescence (Ex 285 nm, Em 310 nm). Quantification of both HPLC assays was achieved by integration of peak area and comparison with authentic metabolite standards. All microsomal incubations were performed at microsomal protein and substrate concentrations yielding linear reactions over the incubation time. Substrate concentrations were 2 μM for all the alkoxyresorufins, 25 μM for 7-ethoxy-4-trifluoromethylcoumarin, 40 μM for bufuralol and 200 μM for testosterone. Protein concentrations were 0.5 mg/ml for testosterone, 7-ethoxy-4-trifluoromethylcoumarin and 7-pentoxyresorufin incubations, 0.2 mg/ml for bufuralol and 0.05 mg/ml for 7-methoxy-, 7-ethoxy- and 7-benzoxoresorufin reactions.

2.4. Hepatic PCB concentrations

Liver pieces (~150 mg) were homogenized in 4 volumes of normal saline and an internal standard, triphenylmethane added proportionate to wet tissue weight. The mix was extracted 3 times with 4 volumes of ethyl acetate, and the combined extracts evaporated to dryness. The residue was redissolved in 100 μl of acetonitrile and 50 μl subjected to HPLC analysis (Discovery C18, $25 \times 4.6 \text{ mm}$, 5 μm column) at 35°C with increasing acetonitrile concentrations (30 to 70% in 0–5 min, 70% for 35 min, 70–99% over 40–45 min, 99% held for 20 min), monitoring absorbance at 220 nm. Chromatography conditions leaned heavily on prior information from the analysis of commercial samples of Aroclor 1254 [17,18]. PCB peak areas (each containing multiple components) eluting at 25, 27, 30, 32, 35, and 39 min were normalized to the internal standard, which eluted at 14.8 min. Strongly absorbant (220 nm) endogenous components eluted between 16 and 24 minutes. Typical elution profiles of ethyl acetate tissue extracts are shown in Figure 1.

2.5. Statistics

Statistical analyses were undertaken using ANOVA and group differences were assessed by Fisher's Partial Least Squares Difference multiple range test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Hepatic mono-oxygenase activities

The response of hepatic cytochrome P450 activities to a single injection of Aroclor 1254 or 3MC was examined over five days in wild-type and *Cyp1a2*($-/-$) mice. Following 3MC administration, 7-ethoxyresorufin (EROD) and 7-ethoxy-4-trifluoromethylcoumarin deethylation (ETCD) activities were no different between wild-type and *Cyp1a2*($-/-$) animals (Figure 2, left panels). Both these activities responded similarly, reaching peak induction at 48 hr and decreasing thereafter. However, after receiving Aroclor 1254 (Figure 2, right panels), both activities were induced to a much higher extent in the *Cyp1a2*($-/-$) mice at every time point examined. By five days, ETCD activity in *Cyp1a2*($-/-$) animals was more than double that of wild-type while 7-ethoxyresorufin deethylase activity was triple that of wild-type. The baseline PROD activity was lower in *Cyp1a2*($-/-$) mice and this differential was maintained during induction by both 3MC and Aroclor 1254 (Figure 3). As with the previous activities, the less persistent 3MC elicited a peak induction response at 48 hr, whereas induction following Aroclor 1254 increased continuously over 5 days. MROD, and to a lesser extent, 7-benzoxoresorufin dealkylation (BROD) activities also had lower constitutive activity in *Cyp1a2*($-/-$) animals, but when considered together with the induction time course values, these did not achieve statistical significance (Figure 4). The *Cyp1a2*($-/-$) < wild-type differential was maintained during induction by 3MC, which in both genotypes showed maximal induction at 48 hr (Figure 4; left panels). With Aroclor 1254, however, *Cyp1a2*($-/-$) mice showed accelerated induction, with activities markedly elevated compared to wild-type after 48 hr, but reaching the same level at 5 days (Figure 4; right panels). Bufuralol 1'-hydroxylation was the only cytochrome P450 activity investigated that showed a significant sex difference (female > male) in naïve animals (Table 1). In neither sex was there a genotype difference in naïve animals, and induction by either 3MC or Aroclor 1254, if any, tended towards only modest increases (up to ~50%). The weak induction was variable between sex and genotype. For example, in wild-type mice, only Aroclor 1254 induced activity and only in females (50% increase). In *Cyp1a2*($-/-$) mice, only males were induced, 41% by Aroclor 1254, 51% by 3MC. Testosterone 6 β -hydroxylase activity was slightly higher (16%) in *Cyp1a2*($-/-$) mice compared to wild-type animals (Table 2). At 48 hr after 3MC or Aroclor 1254, this activity showed a tendency towards minor decreases in both genotypes, more so in *Cyp1a2*($-/-$) than wild-type, and more so with 3MC than with Aroclor 1254.

3.2. Cytochrome P450 characteristics

All of the changes in activities occurred against a backdrop of similar overall cytochrome P450 concentration in naïve *Cyp1a2*($-/-$) and wild-type mice (Table 2). In the induction of cytochrome P450, Aroclor 1254 at 48 hr caused a 24% increase in concentration with a ~1 nm hypsochromic shift in the ferrous-CO maximum in wild-type, compared to no change in concentration and no hypsochromic shift in *Cyp1a2*($-/-$) mice. Induction changes with 3MC were also greater in wild-type with a 60% increase in concentration and a ~2 nm hypsochromic shift versus a 10% increase and a ~1 nm hypsochromic shift in *Cyp1a2*($-/-$) mice.

3.3. Liver PCB concentrations

To investigate whether the absence of *Cyp1a2* might influence the metabolism, and in turn, the accumulation of Aroclor 1254 components in the liver, liver samples taken 48 hr after injection were extracted with ethyl acetate and subjected to HPLC analysis (Figure 1 and Table 3). Six clearly distinguishable, but broad multi-component PCB peaks were identified and while all were lower in *Cyp1a2*($-/-$) animals, only one, the peak eluting maximally at 35 minutes showed a statistical difference ($p < 0.04$) between *Cyp1a2*($-/-$) and wild-type mice. The area of this peak was 37% lower in *Cyp1a2*($-/-$) mice. Summation of all the PCB peak areas showed an overall 34% lower level in *Cyp1a2*($-/-$) animals.

4. Discussion

The study demonstrates that deletion of one cytochrome P450 can significantly alter the induction response of mono-oxygenase activities semi-selective for other P450s. Specifically, the study has demonstrated that various mono-oxygenase activities can be unchanged, induced but to lower levels, induced to the same extent, or induced to higher levels in *Cyp1a2*($-/-$) mice relative to wild-type in response to 3MC and the mix of PCBs present in Aroclor 1254. A limiting feature of the results is an ability to attribute the mono-oxygenase activities variously affected to specific mouse isoforms. This limitation arises from a paucity of available information, the promiscuity of the isoforms for the substrates, and some contrary findings between microsomes and expressed enzymes. A possible example of apparent contrary findings can be found among the alkoxyresorufins where *Cyp1a2* metabolizes only methoxy- and ethoxyresorufin [15] yet pentoxyresorufin dealkylation activity is lower in hepatic microsomes from *Cyp1a2*($-/-$) animals, compared to wild type (Figure 2). This lower PROD activity in *Cyp1a2*($-/-$) animals was evident throughout induction of this activity by either 3MC or Aroclor 1254, suggesting that *Cyp1a2* could be responsible for some of this activity in wild type animals. An alternative explanation that would accommodate the observations, however, is that in some manner, in the absence of *Cyp1a2*, expression of *Cyp1b1* and/or *Cyp2b20*, the two enzymes to which PROD activity has been attributed [15] is suppressed. These two enzymes, together with *Cyp1a1*, also contribute to BROD activity, and constitutive, and throughout the induction time course for 3MC, BROD activity is suppressed in *Cyp1a2*($-/-$) animals, thereby lending some credence to the suppression conjecture. That BROD activity is not lower in *Cyp1a2*($-/-$) mice with Aroclor 1254 induction could indicate that component(s) or effects of Aroclor 1254 not present with 3MC can overcome or are not influenced by the suppression. The similarity in induction responses of MROD and BROD activities, and the observation that the major difference in the enzymes that contribute to the two activities resides with no contribution of *Cyp2b20* to MROD activity [15], would suggest that the expression of *Cyp1b1* is the response selectively influenced by Aroclor 1254.

In contrast to the hypo-induction in *Cyp1a2*($-/-$) mice of MROD, PROD and BROD activities with 3MC administration, EROD and ETCD activities were induced similarly, bufuralol 1'-hydroxylation was only slightly elevated with a tendency to greater elevation in *Cyp1a2*($-/-$) mice, and testosterone 6 β -hydroxylation was slightly decreased with a tendency to greater depression in *Cyp1a2*($-/-$) mice. Thus any influence of the absence of *Cyp1a2* varies with the activity (enzymes) being monitored, and is not a generalized phenomenon on the induction process *per se*.

Following Aroclor 1254 treatment, and in contrast to 3MC effects, EROD and ETCD activities were induced to a greater extent in *Cyp1a2*($-/-$) mice (Figure 2). This observation of hyper-induction in animals lacking one enzyme and the possibly suppression of others raised the possibility that with metabolism and elimination compromised, these mice may have higher levels of PCBs and as a consequence, greater induction. Determination of PCB levels present in the liver at 48 hours revealed an overall reduction (34%) in the amount of PCBs present in *Cyp1a2*($-/-$) animals, with no individual eluting fraction (peak) going counter to the general trend. Only in one fraction, those PCBs eluting at 35 min, was the difference between the two genotypes statistically significant. Late-eluting PCBs likely present in the 35 min peak are the more highly chlorinated components [17]. It may be that the composition of the PCB components present in each fraction, rather than the total differs in the two genotypes. If the components have differing inducing properties, this could influence the induction seen. The mechanism by which the absence of *Cyp1a2* can allow a greater degree of induction therefore awaits elucidation. Although drawing scant attention at the time, data indicating hyper-induction of mono-oxygenase activities, and to a more limited extent immunoreactive cytochrome P450s, in *Cyp1a2*($-/-$) mice has been observed before. After seven weeks of daily

exposure to Aroclor 1254 (0.01% in the diet), *Cyp1a2*($-/-$) mice showed higher immunoreactive Cyp1a1 and Cyp2b10 levels than wild-type animals [14]. Five weeks after a single dose of TCDD, EROD activity and Cyp1a1 immunoreactive protein, were higher in *Cyp1a2*($-/-$) animals [5]. MROD activity in these TCDD-treated animals was higher in wild-type animals. Induction by 3MC has also been examined previously in *Cyp1a2*($-/-$) mice. Although Kondraganti et al., [19] and Sinclair et al., [13] found EROD activity to be induced to similar levels in wild-type and *Cyp1a2*($-/-$) animals, 1 and 8 days after 4 daily doses of 3MC (27 mg/kg) and 2 days after a single injection of 100 mg/kg, respectively, an earlier study [12] had seen induction of this activity to much higher levels in *Cyp1a2*($-/-$) animals 2 days after a single dose of 75 mg/kg. The studies also differed with respect to observations on MROD activity, with the earliest study showing induction to similar levels in the two genotypes but induction to much lower levels in *Cyp1a2*($-/-$) animals in the later studies.

In studies by Sinclair et al., [12,13] and Smith et al., [5], constitutive activity of MROD but not EROD was lower in *Cyp1a2*($-/-$) animals. Our study has a similar finding. The presence of even some MROD activity in *Cyp1a2*($-/-$) mice serves to underscore that this activity in mice is catalyzed by enzymes in addition to Cyp1a2, a conclusion reached by Hamm et al., [20] and recently reinforced by the study of McLaughlin et al., [15].

Constitutive ETCD and bufuralol 1'-hydroxylation activities were little different in the two genotypes. ETCD is associated with CYP2B6 in humans [21] but appears not to be associated with this subfamily in mice, since it has a better correlation with the Cyp2D subfamily and to a lesser extent the Cyp3A subfamily [22]. Bufuralol 1'-hydroxylation activity in mice correlated with a female-dominant mouse cytochrome P450, which was detected with antibodies against rat CYP2D4. Testosterone 6 β -hydroxylation in naive mice is well correlated with the Cyp3A subfamily [22]. Testosterone 6 β -hydroxylase activity was slightly, but statistically significant, higher in *Cyp1a2*($-/-$) mice. While the cytochrome P450 2D subfamily assignment of bufuralol metabolism fits well with the absence of major induction by the Ah receptor agonist, 3MC, the ~8-fold induction of ETCD by 3MC indicates that other P450s in addition to 2D subfamily members can contribute to this reaction. That the induction is the same in wild-type and *Cyp1a2*($-/-$) mice indicates that Cyp1a2 is not amongst them. Without identification of specific substrates for mouse Cyps, or without mouse strains containing multiple null Cyp alleles, explanations for induction phenomenon in laboratory animal species based on enzyme activities will likely remain ill-defined.

The lower activity levels reached for MROD, PROD and BROD activities in *Cyp1a2*($-/-$) mice compared to wild-type following 3MC exposure can most easily be interpreted as loss due to the absence of the Cyp1a2 contribution. Aroclor 1254 consists of a mixture of PCBs, some of which are coplanar and can function as Ah receptor agonists much like 3MC, and others which are not coplanar and function as inducers by other mechanisms. Highly chlorinated biphenyls, for example, activate rodent (rat and mouse) pregnane X receptors [23] and many others can act as "phenobarbital-type" inducers [24]. If these other mechanisms make a considerable contribution to induction of activities the loss of the *Cyp1a2*($-/-$) contribution might pass without notice. However, this would not account for the observed hyper-induction seen with Aroclor 1254 in *Cyp1a2*($-/-$) mice. The presence of higher concentrations of PCBs that induce the enzymes responsible for the hyper or accelerated induction activities was deemed an unlikely explanation given the lower levels of total PCBs and all individually eluting PCB fractions. Lower levels of PCBs could account for apparent hyper induction since it has been shown that tetrachlorobiphenyls can inhibit rat microsomal alkoxyresorufin dealkylase activities [25]. However the dealkylase inhibition profile of tetrachlorobiphenyls based on planarity would pair EROD with MROD activities and separate BROD activity, rather than the pairing of MROD and BROD and separation of EROD activity shown in the present study. A possible explanation for apparent hyper-induction could be the

loss in *Cyp1a2*($-/-$) animals of a *Cyp1a2*-dependent generation from Aroclor 1254 components of an enzyme inhibitor that survives microsome preparation; an inhibitor that is selective for those reactions showing hyper-induction. However we could find no evidence of mouse *Cyp1a2* involvement in PCB metabolism, nor, in a study with a single PCB isomer (PCB153) is it linked to hepatic sequestration [26]. A third possible explanation for this observation is that PCB metabolites generated by *Cyp1a2* interfere with the induction process. It has been observed, for example, that a hydroxypentachlorobiphenyl is a potent suppressor of thyroid hormone (T3)-mediated transactivation [27]. If a similar suppressor effect were operative for the induction mechanisms of those cytochrome P450s contributing to the reactions showing hyper-induction, induction in wild-type could be suppressed by metabolites generated by *Cyp1a2* while induction would proceed unsuppressed and to higher levels in *Cyp1a2*($-/-$) mice. Enhanced or hyper-induction is not seen with the Ah receptor agonist, 3MC, indicating that either this compound does not provide an induction suppressor, or the enhanced induction with Aroclor 1254 occurs through a mechanism other than the AhR/XRE pathway. Much is not known about the differential targeting of cytochrome P450 subfamilies for proteasomal degradation, but loss of a *Cyp1a2*-dependent downstream effect that retards degradation of enzymes showing hyper-induction could also contribute to the phenomenon. The several explanations offered underscore the existence of the multiple transcriptional, translation and post-translational effects known to affect the activity of the cytochrome P450 drug metabolizing system.

Whatever the mechanism, or whether a combination of mechanisms are operating, it is clear that deletion of one cytochrome P450 subfamily member influences the induction response, as revealed by enzyme activity, of other enzymes. Unfortunately, the promiscuity or lack of substrate specificity of mouse cytochrome P450s, as clearly documented by McLaughlin et al., [15] and reinforced with different substrates in the present study precludes absolute identification of the enzyme(s) so affected. As has been found so often, the knockout of one enzyme (in this study, *Cyp1a2*) can have multiple downstream consequences to biochemical processes (in this study, enzyme activity increases in response to inducers) not previously associated with the catalytic activity of the deleted enzyme. While complete deletion of a cytochrome P450 may be a rare scenario in humans, polymorphisms resulting in much decreased or less often complete loss of enzyme activity are known and these could affect the activity of other P450s as has been modeled in the present study with gene deletion in mice.

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Figure 1. HPLC elution profiles of ethyl acetate extracts of mouse livers

HPLC elution traces are of ethyl acetate extracts from the liver of a mouse receiving Aroclor 1254 48 hr prior (top), the liver of an untreated (naïve) mouse to which has been added Aroclor 1254 in vitro (center) and the liver of an untreated mouse (bottom). Internal standard (IS) and major elution peaks attributable to PCBs are elution-time labeled in the top trace.

7-Ethoxy-4-trifluoromethylcoumarin deethylation

7-Ethoxyresorufin deethylation

Figure 2. Effect of 3-methylcholanthrene (left) and Aroclor 1254 (right) on 7-ethoxy-4-trifluoromethylcoumarin (top) and 7-ethoxyresorufin (bottom) dealkylations in wild-type (solid symbols) and *Cyp1a2*^{-/-} (open symbols) mice

Values are mean $\pm$ sem. Group size for 0, 24, 48, 72 and 120 hr after 3-methylcholanthrene (left) was 9, 4, 6, 6 and 4 for wild-type (solid symbols) and 3, 3, 6, 6 and 3 for *Cyp1a2*^{-/-} (open symbols). Group size for 0, 24, 48, 72 and 120 hr after Aroclor 1254 (right) was 11, 3, 6, 4 and 5 for wild-type (solid symbols) and 11, 2, 6, 4 and 4 for *Cyp1a2*^{-/-} (open symbols). Statistically significant differences ($p < 0.05$) between *Cyp1a2*^{-/-} and wild-type mice are indicated with an *.

Activities at all times after inducer administration were significantly elevated above 0 time.

7-Pentoxoresorufin dealkylation

Figure 3. Effect of 3-methylcholanthrene (left) and Aroclor 1254 (right) 7-pentoxoresorufin dealkylation in wild-type (solid symbols) and *Cyp1a2*(-/-) (open symbols) mice

Values are mean $\pm$ sem. Group sizes are identical to those in Figure 2. Statistically significant differences ($p < 0.05$) between *Cyp1a2*(-/-) and wild-type mice are indicated with an *. Activities at all times after inducer administration were significantly elevated above 0 time.

7-Methoxyresorufin demethylation

7-Benzoxoresorufin dealkylation

Figure 4. Effect of 3-methylcholanthrene (left) and Aroclor 1254 (right) on 7-methoxy-(top) and 7-benzoxoresorufin (bottom) dealkylations in wild-type (solid symbols) and *Cyp1a2*(-/-) (open symbols) mice

Values are mean $\pm$ sem. Statistically significant differences ($p < 0.05$) between *Cyp1a2*(-/-) and wild-type mice are indicated with an *.

Activities at all times after inducer administration were significantly elevated above 0 time.

Table 1

Effect of 3-methylcholanthrene and Aroclor 1254 on hepatic bufuralol 1'-hydroxylation in wild-type and *Cyp1a2* (−/−) mice after 48 hours.

	Bufuralol 1'-hydroxylation (pmol/mg/min)		
	Naïve	3-Methylcholanthrene	Aroclor 1254
Male			
Wild-type	70.1 ± 3.8 (7)	87.1 ± 0.5 (3)	68.2 ± 6.9 (3)
<i>Cyp1a2</i> (−/−)	71.5 ± 4.7 (7)	108.5 ± 6.6 (3) ^b	100.8 ± 10.4 (3) ^b
Female			
Wild-type	100.0 ± 6.9 (6) ^a	107.7 ± 2.2 (3)	150.2 ± 12.9 (4) ^{a,b}
<i>Cyp1a2</i> (−/−)	108.0 ± 6.0 (7) ^a	129.6 ± 9.3 (3)	107.1 ± 6.0 (3)

Values are mean ± sem, followed in parentheses by the number of individual animals in the group.

^a significant (p < 0.05) sex difference.

^b significant induction (p < 0.05) over naïve of same genotype and sex.

Table 2

Effect of Aroclor 1254 and 3-methylcholanthrene on hepatic cytochrome P450 concentration and testosterone 6 β -hydroxylation in wild-type and *Cyp1a2*($-/-$) mice after 48 hours.

	P450 nmol/mg	CO max (nm)	Testosterone 6 β -hydroxylation (nmol/mg/min)
Wild-type			
Naïve	0.495 $\pm$ 0.024 (13)	450.1	5.49 $\pm$ 0.28 (15)
Aroclor 1254	0.616 $\pm$ 0.055 (10) ^a	448.9	5.21 $\pm$ 0.33 (10)
3-Methylcholanthrene	0.791 $\pm$ 0.027 (5) ^a	448.1	4.72 $\pm$ 0.23 (6)
<i>Cyp1a2</i> ($-/-$)			
Naïve	0.535 $\pm$ 0.037 (10)	450.0	6.37 $\pm$ 0.46 (15) ^b
Aroclor 1254	0.536 $\pm$ 0.017 (6)	450.1	5.68 $\pm$ 0.48 (6)
3-Methylcholanthrene	0.587 $\pm$ 0.024 (6) ^b	449.1	4.52 $\pm$ 0.37 (6)

Values are mean $\pm$ sem, followed in parentheses by the number of animals comprising the group.

^a significant difference ($p < 0.05$) from untreated animals of the same genotype.

^b significantly elevated ($p < 0.05$) above wild-type of the same treatment.

Table 3

Hepatic PCB concentrations in wild-type and *Cyp1a2*($-/-$) mice 48 hours after Aroclor 1254 administration.

HPLC Retention time (min)	PCB peak area (peak $\times$ 1000/IS)	
	Wild-type	<i>Cyp1a2</i> $-/-$
25	72 $\pm$ 25	47 $\pm$ 19
27	592 $\pm$ 82	406 $\pm$ 53
30	559 $\pm$ 105	401 $\pm$ 63
32	311 $\pm$ 57	180 $\pm$ 30
35	236 $\pm$ 37	148 $\pm$ 22 ^b
39	88 $\pm$ 25	41 $\pm$ 18

Values are mean $\pm$ sem from 6 mice of each genotype assayed independently.

^a Naïve liver background subtracted.

^b Significantly different vs. wild-type ($p < 0.05$)