Biochemical Pharmacology, Vol. 35, No. 20, pp. 3495–3500, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

MAJOR DIFFERENCES BETWEEN LUNG, SKIN AND LIVER IN THE MICROSOMAL METABOLISM OF HOMOLOGOUS SERIES OF RESORUFIN AND COUMARIN ETHERS*

ALLAN E. RETTIE,†‡ FAITH M. WILLIAMS,† MICHAEL D. RAWLINS,† RICHARD T. MAYER§ and M. DANNY BURKE‖¶

†Wolfson Unit of Clinical Pharmacology, Claremont Place, University of Newcastle-Upon-Tyne NE1 7RU, U.K.; § Horticultural Research Laboratory, USDA, Orlando, Fl 32803, U.S.A.; ‖ Department of Pharmacology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

(*Received* 15 *November* 1985; *accepted* 18 *April* 1986)

Abstract—Phenoxazone and a homologous series of its ethers (methoxy to octoxy plus benzyloxy), and coumarin and a series of its ethers (methoxy to propoxy), were metabolized by liver, lung and skin microsomes of normal adult female BALB/c mice. For each series of substrates, and with each tissue, clear structure–activity relationships were seen, relating metabolic activity to the length of the ether side-chain. With the coumarin series of substrates the structure–activity relationships were almost identical in the three tissues, with liver more active than lung and lung more active than skin. Liver, lung and skin microsomes each showed very different structure–activity relationships, however, for metabolism of the phenoxazone series of substrates. Benzyloxyphenoxazone was metabolized almost twice as fast in lung as in liver, but for the other phenoxazone substrates the activities were much greater in liver than in lung or skin. Liver, lung and skin microsomal propoxy- and benzyloxyphenoxazone dealkylase activities differed in their sensitivities to inhibition by metyrapone and α-naphthoflavone. The structure–activity relationship and inhibitor data for the phenoxazone substrates are consistent with a view that mouse lung and sking cyt. P-450 are predominantly similar to phenobarbitone-induced and 3-methylcholanthrene-induced forms of hepatic cyt. P-450 respectively. The results also show that the pattern of microsomal metabolism of xenobiotics in lung and skin cannot be reliably predicted from that in liver.

Both pulmonary and cutaneous tissues are primary targets for the toxic effects of chemicals. The pulmonary necrosis caused by 4-ipomeanol and 3-methylfuran [1, 2] and several examples of skin tumourigenesis initiated by topically applied chemical carcinogens [3] are processes requiring bioactivation of the toxic chemicals by cyt. P-450-dependent monooxygenase enzymes. Since in many cases the organ-specific nature of chemical toxicity may reflect the balance of metabolic activation and detoxification in the target tissue [4], the concentrations, specificities and activities of different forms of cyt. P-450 in target tissues may be a major determinant of toxicity. Characterisation of cytochromes P-450 in target tissues is, therefore, of considerable importance. Whilst several forms of hepatic cyt. P-450 have been purified and extensively characterized [5, 6], there is much less information available about extrahepatic cyt. P-450. We report here a study into the comparative substrate specificities of hepatic, pulmonary and cutaneous cyt. P-450, using two different homologous series of heterocyclic ether substrates, for which different forms of hepatic cyt. P-450 show differing selectivities. A number of phenoxazone ethers, homologous with ethoxyresorufin, show clearly differing substrate selectivities for different induced forms of rat and mouse liver cyt. P-450 [7, 8]. The 1C to 3C alkyl ethers of coumarin are substrates which, by their selectivity, distinguish to some extent between different induced and apparently gender-specific forms of cyt. P-450 in rats [9–12].

MATERIALS AND METHODS

Chemicals. Phenoxazone, benzyloxyphenoxazone and the homologous series of 1C–8C *n*-alkyl ethers of phenoxazone were synthesized and their structures confirmed as described previously [7]. Resorufin was purchased from Molecular Probes Inc. (Junction City, Oregon 97448, U.S.A.) and several of the ethers also can be obtained from this company. Coumarin, 7-hydroxycoumarin, NADH and NADPH were purchased from the Sigma Chemical Co. (Poole, Dorset). The 1C–3C *n*-alkyl ethers of coumarin were prepared by reaction of 7-hydroxycoumarin with the appropriate alkyl iodide and their structures confirmed by nuclear magnetic resonance spectroscopy and mass spectrometry, as described elsewhere [13]. ANF was purchased from the Aldrich Chemical Co. (Gillingham, Dorset), whilst sodium phenobarbitone and metyrapone were obtained from

* The mention of a proprietary product in this article does not constitute an endorsement by the United States Department of Agriculture.

‡ Present address, Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195, U.S.A.

¶ Author to whom all correspondence should be addressed.

BDH Ltd. (Poole, Dorset). 3MC was purchased from Fluka AG (Buchs, Switzerland).

Animals. Female BALB/c mice (20–25 g) were bred and housed in the Medical School Animal Unit of the University of Newcastle-upon-Tyne. The mice were subjected to a constant 12 hr artificial light cycle, bedded on wood shavings and allowed free access to food (mouse diet no. 41D (Glaxo), supplied by Styles Ltd., Worcester) and water. Phenobarbitone (100 mg.kg^{-1} of the sodium salt in saline) was administered by i.p. injection for 3 days, whilst 3MC* (100 mg.kg^{-1} in corn oil) was given i.p. once only. Mice were killed 24 hr after the last injection of either agent.

Preparation of microsomes. Groups of 12 mice were killed by cervical dislocation. All subsequent procedures were carried out at 4°. 10% (w/v) homogenates of liver, lung and skin pooled from 12 mice were prepared in 0.1 M phosphate buffer (pH 7.5, containing 0.15 M KCl). Liver (minus the gall bladder) and whole lung (including bronchial tissue) were finely minced with scissors and homogenized using sequentially a Polytron Homogeniser (PT10S with a PTA10S head; Kinematica, Switzerland) for 5 sec at maximum speed, followed by a Teflon–glass Potter-Elvejhem-type homogenizer (25 ml size, A. H. Thomas Co., Philadelphia, U.S.A.). For the preparation of skin the mice were first shaved using an electric clipper. Skin was cut from the entire body up to the neck and subcutaneous fat was removed using the trailing edge of a scalpel blade. The skin was then finely minced with scissors, frozen in liquid nitrogen and pulverized using a mortar and pestle. Approximately 1.5 g of powdered skin was homogenized in 12 ml 0.1 M phosphate buffer (pH 7.5 containing 0.15 M KCl), using two 10-sec bursts of a Polytron Homogeniser at maximum speed and the crude skin homogenate was filtered through 2 layers of gauze. Homogenates of each organ were centrifuged for 5 min at 1000 *g* followed by 10 min at 18,000 *g*, using a Sorvall RC-5B centrifuge. Skin post-mitochondrial supernatant was centrifuged for 10 min at 18,000 *g* to prevent gross lipid contamination of the microsomes. Microsomes of each organ were prepared by centrifugation of the 18,000 *g* supernatant for 60 min at 120,000 *g* using a Sorvall OTD-65B ultracentrifuge. The microsomal pellets from liver and skin were resuspended in 0.01 M phosphate buffer (pH 7.5, containing 0.15 M KCl) at concentrations of 1–3 mg protein.ml^{-1}. Lung microsomal pellets were resuspended in 0.1 M phosphate buffer (pH 7.5, containing 0.15 M KCl) and recentrifuged for 40 min at 186,000 *g* to remove haemoglobin and the washed microsomes resuspended as for liver and skin. All experiments were carried out using freshly prepared microsomes. The liver, lungs and skin were obtained from the same pooled groups of mice.

Enzyme assays. The 7-hydroxylation of phenoxazone and the 7-*O*-dealkylation of the phenoxazone ethers were measured using the continuous fluorimetric assay of Burke and Mayer [7], with the following modifications: reactions were carried out in a microcuvette for 1–5 min at 24° in a final volume of 0.5 ml, comprising 0.01 M phosphate buffer (pH 7.5, containing 0.15 M KCl), 3 mM $MgCl_2$, 0.8 mM NADPH, 0.8 mM NADH and 2 μM substrate. Metabolic formation of resorufin (7-hydroxyphenoxazone) was measured continuously using a Perkin-Elmer MPF-3 spectrofluorimeter with excitation and emission wavelengths at 530 nm and 585 nm respectively. Each assay was performed in duplicate and initial reaction velocities calculated after fluorescence calibration by the addition of a known amount of authentic resorufin to the cuvette at the end of the reaction period. The 7-hydroxylation of coumarin and the 7-*O*-dealkylation of the coumarin ethers were assayed by the end-point fluorimetric method of Greenlee and Poland [14], using excitation and emission wavelengths of 370 nm and 456 nm respectively. Reactions were carried out at 37° with 500 μM substrate, for 10 min in the case of liver and for 20 min in the case of lung and skin. Assays were carried out in triplicate and initial reaction velocities calculated by comparison with the fluorescence of known concentrations of authentic 7-hydroxycoumarin extracted from other samples of microsomes (recovery of 7-hydroxycoumarin was 79 ± 2%). The amounts of microsomal proteins added to the reactions with either series of substrates were chosen to ensure initial velocity conditions over the period of measurement. With the phenoxazones the amounts needed varied greatly, depending on the organ and the induction status, and were easily and quickly found by trial and error. With the coumarins the amounts were approximately 0.5 mg for liver and approximately 1.0 mg for lung and skin. Protein concentrations were measured by the method of Miller [15].

Inhibition studies. Either ANF or metyrapone were added to incubations *in vitro* in 5 μl DMSO, to give final concentrations of either 1, 10 or 100 μM. DMSO (5 μl) was added to all controls, but was found to have no significant effect on the reactions.

RESULTS

Metabolism of phenoxazone and its ethers by microsomes from untreated mice

Phenoxazone and its various ethers were each metabolized at a different rate by liver microsomes from untreated mice. Resorufin was the product with each substrate, formed by the *O*-dealkylation of the ethers or the ring-hydroxylation of the parent compound. Plotting the reaction rates against the chain length of the metabolized ether sidechain gave a structure–activity relationship that was characteristic for liver (Fig. 1). Similar observations were made with lung and skin microsomes, but the reaction rates and the structure–activity relationships were very different between liver, lung and skin microsomes (Fig. 1). The preferential metabolism of the benzyl ether by lung microsomes is especially noticeable. With skin microsomes phenoxazone and methoxyphenoxazone were much poorer substrates than ethoxyphenoxazone, whereas with liver microsomes all three compounds were metabolized at similar

* Abbreviations used: ANF, α-naphthoflavone; BNF, β-naphthoflavone; 3MC, 3-methylcholanthrene; PB, phenobarbitone.

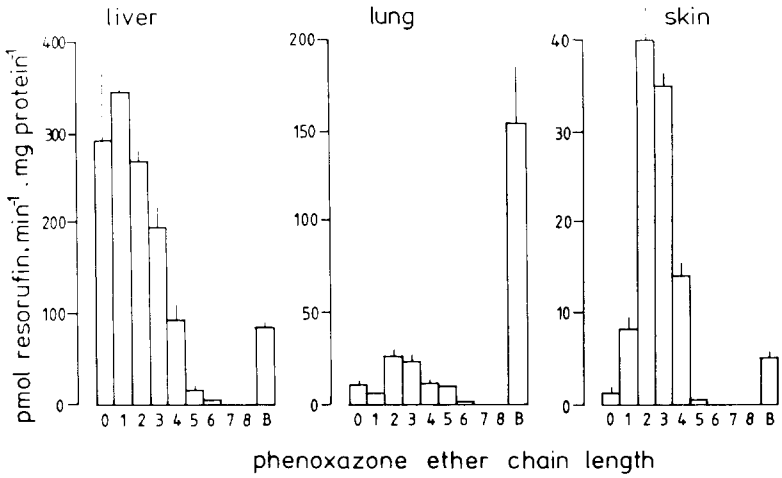

Fig. 1. Structure–activity relationships for the metabolism of phenoxazone and a homologous series of its ethers by liver, lung and skin microsomes from untreated mice. The numbers below the bars indicate the length of the metabolized ether side-chain (e.g. 0 = the parent compound, phenoxazone; 2 = ethoxy; B = benzyloxy). Results are means ± SEM for 3 separate experiments, i.e. microsomes from 3 different pooled groups of mice.

rates. The lung specific activities for metabolism of all but two of the phenoxazone substrates were less than 15% of the corresponding liver activities, but for pentoxy- and benzyloxyphenoxazone the lung activities were 67% and 182% of the liver activities respectively. The skin activities for all the phenoxazone substrates were less than 20% of the liver.

Inhibition of the metabolism of propoxy- and benzyloxy-phenoxazone

Ethoxy- and propoxyphenoxazone were the best phenoxazone substrates with skin microsomes, whilst benzyloxyphenoxazone was the best with lung microsomes. Propoxy- and benzyloxyphenoxazone are selective substrates for 3MC-induced and PB-induced forms of rat liver cyt. P-450 respectively [8]. In this study propoxyphenoxazone metabolism by liver microsomes was induced 33-fold by 3MC but only 2-fold by PB (Table 1), whereas benzyloxyphenoxazone metabolism by liver microsomes was induced only 4-fold by 3MC but 19-fold by PB (Table 2). ANF and metyrapone are selective inhibitors of 3MC-induced and PB-induced forms of hepatic cyt. P-450 respectively [16]. Tables 1 and 2 show the inhibition of the metabolism of propoxy- and benzyloxyphenoxazone by ANF or metyrapone *in*

Table 1. Inhibition of microsomal propoxyphenoxazone *O*-dealkylation by α-naphthoflavone or metyrapone

Concn. (μM)*	Liver Untreated†	Liver PB	Liver 3MC	Lung Untreated	Skin Untreated
ANF 1	65 ± 3‡	47 ± 2	12 ± 2	15 ± 2	36 ± 3
ANF 10	37 ± 1	36 ± 3	0	0	2 ± 1
ANF 100	12 ± 1	12 ± 1	0	0	0
MET 1	87 ± 2	82 ± 4	103 ± 3	96 ± 5	100 ± 4
MET 10	80 ± 4	75 ± 5	97 ± 5	97 ± 4	88 ± 5
MET 100	78 ± 3	55 ± 5	89 ± 2	79 ± 4	96 ± 3
100% values (inhibitor absent: pmol/min/mg protein)§					
	195 ± 20	420 ± 43	6390 ± 690	24 ± 4	35 ± 2

* Either α-naphthoflavone (ANF) or metyrapone (MET) were present in the reaction at the concentrations shown.

† Microsomes were prepared from untreated mice or from mice pretreated with either phenobarbitone (PB) or 3-methylcholanthrene (3MC).

‡ Activities are shown as percentages of control, i.e. the activity measured in the absence of inhibitor.

§ Specific activities are shown for each of the 5 types of microsomes measured in the absence of either ANF or MET.

Table 2. Inhibition of microsomal benzyloxyphenoxazone *O*-dealkylation by α-naphthoflavone or metyrapone

Concn. (μM)	Liver Untreated†	Liver PB	Liver 3MC	Lung Untreated	Skin Untreated
ANF 1	85 ± 2‡	269 ± 61	52 ± 6	98 ± 3	103 ± 6
ANF 10	87 ± 4	292 ± 21	19 ± 2	84 ± 2	93 ± 8
ANF 100	47 ± 10	98 ± 6	7 ± 1	58 ± 7	75 ± 3
MET 1	73 ± 4	70 ± 7	100 ± 3	56 ± 2	65 ± 1
MET 10	40 ± 2	34 ± 2	79 ± 4	15 ± 2	14 ± 2
MET 100	17 ± 1	11 ± 3	38 ± 1	1 ± 1	1 ± 1
100% values (inhibitor absent: pmol/min/mg protein)§					
	85 ± 5	1646 ± 193	351 ± 40	155 ± 30	5 ± 1

* Either α-naphthoflavone (ANF) or metyrapone (MET) were present in the reaction at the concentrations shown.

† Microsomes were prepared from untreated mice or from mice pretreated with either phenobarbitone (PB) or 3-methylcholanthrene (3MC).

‡ Activities are shown as percentages of control, i.e. the activity measured in the absence of inhibitor.

§ Specific activities are shown for each of the 5 types of microsomes measured in the absence of either ANF or MET.

vitro. Propoxyphenoxazone metabolism by control liver, lung and skin microsomes and by PB- or 3MC-induced liver microsomes was much more sensitive to ANF than to metyrapone, although in the case of liver ANF was a stronger inhibitor of 3MC-induced than PB-induced or control microsomes (Table 1). Benzyloxyphenoxazone metabolism by control liver, lung and skin microsomes and by PB-induced liver microsomes was more sensitive to metyrapone than to ANF, whilst in 3MC-induced liver microsomes it was more sensitive to ANF than to metyrapone (Table 2).

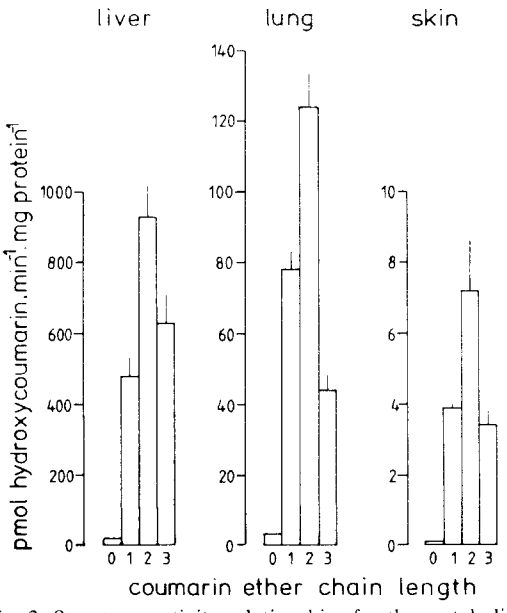

Fig. 2. Structure–activity relationships for the metabolism of coumarin and a homologous series of its ethers by liver, lung and skin microsomes from untreated mice. The numbers below the bars indicate the length of the metabolised ether side-chain (e.g. 0 = the parent compound, coumarin; 2 = ethoxy). Results are means ± SEM for 3 separate experiments, i.e. microsomes from 3 different pooled groups of mice.

Metabolism of coumarin and its ethers by microsomes from untreated mice

Coumarin and its 1C–3C ethers were each metabolized at different rates by liver microsomes from untreated mice. The structure–activity relationship for coumarin ether metabolism was almost identical in liver, lung and skin microsomes (Fig. 2), whereas the structure–activity relationship for phenoxazone ether metabolism was very different with liver, lung and skin microsomes (Fig. 1). For each of the coumarin substrates liver microsomes were more active than lung microsomes, which were in turn more active than skin microsomes.

DISCUSSION

The main finding of this study is that mouse liver, lung and skin microsomes have very different metabolic specificities for a homologous series of phenoxazone substrates but almost identical specificities for a homologous series of coumarin substrates.

The structure–activity relationship reported here for the hepatic microsomal metabolism of phenoxazone and its ethers by untreated female BALB/c mice is almost identical to that reported previously for untreated male C57/BL10 mice [7]. Since the structures of the coumarin and phenoxazone ethers are similar except for being bicyclic and tricyclic respectively, differences in the hepatic structure–activity relationships between the coumarin and phenoxazone substrates suggest an important influence of their ring structure on the specificity of hepatic cyt. P-450. The high rates of metabolism of phenoxazone and methoxyphenoxazone in mice are

in clear contrast to the liver microsomes of untreated male Sprague–Dawley rats, where these two substrates are metabolized at less than 20% of the rate of metabolism of ethoxyphenoxazone [8].

The highly preferential metabolism of the benzyl ether of phenoxazone by lung microsomes is remarkable, especially since the pulmonary specific activity was almost twice the hepatic activity. By contrast, the pulmonary specific activities for the cyt. P-450-mediated metabolism of aminopyrine, aniline and biphenyl in CDF_1 mice are 25% or less of the hepatic activities [17]. In rats also the specific activities of monooxygenase reactions are low in lung compared to liver [17]. In the rabbit [18] and the hamster [19], however, whilst some monooxygenase reactions are very slow in lung compared to liver, the pulmonary specific activities for benzphetamine *N*-demethylation and biphenyl 4-hydroxylation are 43–123% of the hepatic activities. The rabbit is the only species where the pulmonary cyt. P-450 has been extensively researched [18, 20]. The lung microsomal cyt. P-450 of normal rabbits consists almost entirely of two forms, present in almost equal proportions, which are similar or identical to the hepatic microsomal PB-induced Forms 2 and 5 [20, 21]. The high pulmonary activity compared to hepatic activity for the metabolism of benzphetamine is considered to be due to the high proportions of these forms of cyt. P-450 in normal rabbit lung compared to liver [22]. Although cyt. P-450 concentrations were not measured in this study, assuming that the values are similar to those for CDF_1 mice [17], the rate of benzyloxyphenoxazone metabolism per nmol total cyt. P-450 would be approximately 12 times higher in the mouse lung than in the liver. By analogy with rabbit lung, a preponderance in normal mouse lung of a form of cyt. P-450 similar to PB-induced mouse and rat liver P-450 would explain the preference and high activity of mouse lung microsomes for benzyloxyphenoxazone (a selective substrate for PB-induced liver cyt. P-450 [7, 8]) and the preferential inhibition of this reaction in mouse lung microsomes by metyrapone (selective for PB-induced cyt. P-450 [16]) rather than ANF (selective for 3MC-induced cyt. P-450 [16]). This is supported by the fact that the ratio of lung : liver activities was much higher for pentoxy- and benzyloxyphenoxazone (both selective substrates for PB-induced hepatic cyt. P-450, with benzyloxyphenoxazone being the faster [7, 8]) than for the other phenoxazone substrates. However, the preferential inhibition of lung microsomal metabolism of propoxyphenoxazone (a selective substrate of 3MC-induced liver cyt. P-450 [7, 8]) by ANF rather than metyrapone suggests the presence also of a minority of cyt. P-450 forms in normal mouse lung microsomes similar to 3MC-induced liver cyt. P-450.

Moloney *et al.* [23] found that skin microsomes of normal adult male hairless mice metabolized both ethoxyphenoxazone and ethoxycoumarin, but that both reactions were undetectable in skin microsomes of normal young adult male rats. Ethoxycoumarin *O*-deethylase activity was reported present, however, in skin microsomes of normal neonatal rats [24] and in skin whole homogenates of normal adult male BALB/c mice [25].

In our study both ethoxycoumarin and ethoxyphenoxazone *O*-deethylases were active in skin microsomes of untreated mice. Considering just the phenoxazone substrates and using the same arguments put forward in discussing lung microsomes, a preponderance in normal mouse skin microsomes of a form of cyt. P-450 similar to 3MC-induced mouse and rat liver cyt. P-450 would explain the preference of skin microsomes for ethoxy- and propoxyphenoxazone and the preferential inhibition of skin propoxyphenoxazone metabolism by ANF rather than metyrapone. However, the preferential inhibition of skin benzyloxyphenoxazone metabolism by metyrapone rather than ANF suggests also a minority of cyt. P-450 forms in normal mouse skin similar to PB-induced liver cyt. P-450.

Whereas the structure–activity relationship for the metabolism of the phenoxazone series was different in mouse liver, skin and lung microsomes, the structure–activity relationship for the metabolism of the coumarin series was virtually identical in the three tissues, albeit with liver more active than lung and lung more active than skin. This might mean that the coumarin substrates were metabolized by the same form or forms of cyt. P-450 in liver, lung and skin, whereas the phenoxazone substrates were metabolized by different forms of cyt. P-450 in the three tissues. Alternatively it might simply be that the phenoxazone substrates are capable of distinguishing between different forms of murine cyt. P-450, whereas the coumarin substrates are not.

In conclusion, this study shows that patterns of drug metabolism are very different in mouse liver, lung and skin microsomes and that it is not possible to predict reliably the pattern in one tissue from that in another. The results are consistent with a view that lung and skin cyt. P-450 are predominantly similar to PB-induced and 3MC-induced forms of hepatic cyt. P-450 respectively.

REFERENCES

1. M. R. Boyd, *Nature, Lond.* **272**, 270 (1978).
2. M. R. Boyd, J. S. Dutcher, A. R. Buckpitt, R. B. Jones and C. N. Statham, in *Naturally Occurring Carcinogens—Mutagens and Modulators of Carcinogenesis* (Eds. E. C. Miller, J. A. Miller, I. Hirono, T. Sugamura and S. Takayama), pp. 35–56. University Park Press, Baltimore (1979).
3. C. Heidelberger, *Ann. Rev. Biochem.* **44**, 79 (1975).
4. P. Sims, *Br. Med. Bull.* **36**, 11 (1980).
5. A. Y. H. Lu and S. B. West, *Pharmac. Rev.* **31**, 277 (1980).
6. F. P. Guengerich, G. A. Dannan, S. T. Wright, M. V. Martin and L. S. Kaminsky, *Biochemistry* **21**, 6019 (1982).
7. M. D. Burke and R. T. Mayer, *Chem.-Biol. Interact.* **45**, 243 (1983).
8. M. D. Burke, S. Thompson, C. R. Elcombe, J. Halpert, T. Haaparanta and R. T. Mayer, *Biochem. Pharmac.* **34**, 3337 (1985).
9. T. Kamataki, M. Ando, Y. Yamazoe, K. Ishii and R. Kato, *Biochem. Pharmac.* **29**, 1015 (1980).
10. T. Kamataki, K. Maeda, Y. Yamazoe, T. Nagai and R. Kato, *Life Sci.* **31**, 2603 (1982).
11. T. Matsubara, S. Otsubo and E. Yoshihara, *Jap. J. Pharmac.* **33**, 1065 (1983).

12. M. Komori, Y. Imai and R. Sato, *J. Biochem.* **95**, 1379 (1984).
13. A. Rettie, Ph.D. Thesis, University of Newcastle-Upon-Tyne (1983).
14. W. F. Greenlee and A. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
15. G. L. Miller, *Analyt. Chem.* **31**, 964 (1959).
16. F. M. Goujon, D. W. Nebert and J. E. Gielen, *Molec. Pharmac.* **8**, 667 (1972).
17. C. L. Litterst, E. G. Mimnaugh, R. L. Reagan and T. E. Gram, *Drug Metab. Dispos.* **3**, 259 (1975).
18. T. E. Gram, in *Toxicology of Inhaled Materials* (Eds. H. P. Witschi and J. D. Brain), pp. 421–470. Springer, Berlin (1985).
19. M. D. Burke and R. A. Prough, *Biochem. Pharmac.* **25**, 2187 (1976).
20. R. M. Philpot and B. R. Smith, *Environ. Health Perspec.* **55**, 359 (1984).
21. I. G. C. Robertson, C. Seraabjit-Singh, J. E. Croft and R. M. Philpot, *Molec. Pharmac.* **24**, 156 (1983).
22. I. G. C. Robertson, R. M. Philpot, E. Zeiger and C. R. Wolf, *Molec. Pharmac.* **20**, 662 (1980).
23. S. J. Moloney, J. M. Fromson and J. W. Bridges, *Biochem. Pharmac.* **31**, 4011 (1982).
24. D. R. Bickers, T. Dutta-Choudhury and H. Mukhtar, *Molec. Pharmac.* **21**, 239 (1981).
25. F. J. M. Damen and P. D. Mier, *Br. J. Pharmac.* **75**, 123 (1982).