Br. J. clin. Pharmac. (1992), **34**, 415–420

7-alkoxyquinoline *O*-dealkylation by microsomes from human liver and placenta*

JUKKA HAKKOLA[1], JUKKA MÄENPÄÄ[1], RICHARD T. MAYER[2], SANG S. PARK[3], HARRY V. GELBOIN[3] & OLAVI PELKONEN[1]

[1]Department of Pharmacology and Toxicology, University of Oulu, SF-90220 Oulu, Finland, [2]US Department of Agriculture, Agricultural Research Service, US Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803 and [3]Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD 20892, USA

1 The *O*-dealkylation of seven 7-alkoxyquinoline derivatives by human hepatic and placental microsomes and the effect of maternal cigarette smoking on placental 7-alkoxyquinoline metabolism was studied.

2 None of several monoclonal antibodies to isoenzymes of cytochrome P450 had a clear effect on metabolism of the compounds by liver microsomes.

3 Maternal cigarette smoking induced the *O*-dealkylation of all of the 7-alkoxyquinoline derivatives, being greatest for 7-butoxy- and 7-benzyloxyquinoline.

4 Placental 7-alkoxyquinoline metabolism induced by smoking was partially inhibited by the monoclonal antibody 1-7-1 raised against 3-methylcholanthrene-induced rat liver P450.

5 None of the 7-alkoxyquinoline *O*-dealkylations could be assigned specifically to any known P450 isoenzyme in human liver or placenta.

Keywords 7-alkoxyquinoline cytochrome P450 human liver human placenta cigarette smoking

Introduction

The cytochrome P450 system metabolizes many endogenous compounds and xenobiotics (Lu & West, 1980; Nebert *et al.*, 1987). In mammalian species there are multiple forms of P450 which are the products of different genes (Guengerich, 1989; Nebert *et al.*, 1989).

Because P450 isoenzymes have different, but overlapping substrate specificities, one way to characterize different isoenzymes is to determine their 'metabolic fingerprints', i.e. their qualitative and quantitative activity towards different substrates. For example, homologous series of coumarin and resorufin derivatives have been studied and 7-ethoxy- and 7-pentoxyphenoxazones have been shown to be relatively specific substrates for the 3-methylcholanthrene (MC)- and phenobarbitone (PB)-inducible forms of P450, respectively (Burke & Mayer, 1983). The reason for the high substrate specificity of these phenoxazones is unclear. However, the phenoxazones contain both nitrogen and oxygen atoms in the fluorophore ring system, while other, less isoenzyme-specific substrates with fluorophore rings (coumarins) contain only an oxygen atom. 7-Alkoxyquinolines were recently proposed as fluorescent substrates for the assay of microsomal monooxygenases (Mayer *et al.*, 1989, 1990). These compounds contain a nitrogen atom in the aromatic ring and are thus suited for study of its effect on P450 substrate specificity (Figure 1).

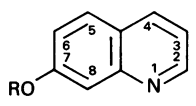
Studies of the metabolism of 7-alkoxyquinolines with variously pre-treated rat and mouse liver (Hahnemann *et al.*, unpublished; Mayer *et al.*, 1989, 1990; Netter *et al.*, 1990) have shown both species and pre-treatment variation. We have studied 7-alkoxyquinoline metabolism by microsomes from human liver and placenta and have also performed immunoinhibition assays using an assay of antibodies.

Previous studies have shown that maternal cigarette smoking induces the placental metabolism of several xenobiotics; this increased activity being mainly due to the induction of the CYP1A1 isoenzyme (Pasanen *et al.*, 1990; Sesardic *et al.*, 1990). In the present study the

Correspondence: Dr O. Pelkonen, Department of Pharmacology and Toxicology, University of Oulu, SF-90220 Oulu, Finland

*Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

P450 nomenclature: The current P450 nomenclature is that recommended by Nebert *et al.* (1991). CYP1A1 is an isoenzyme which belongs to the polycyclic hydrocarbon inducible CYP1A subfamily.



R = — H (7-Hydroxy-)
= — CH_3 (7-Methoxy-)
= — CH_2CH_3 (7-Ethoxy-)
= — $CH_2CH_2CH_3$ (7-Propoxy-)
= — $CH_2(CH_2)_2CH_3$ (7-Butoxy-)
= — $CH_2(CH_2)_3CH_3$ (7-Pentoxy-)
= — $CH_2(CH_2)_4CH_3$ (7-Hexoxy-)
= — $CH_2(CH_6H_5)$ (7-Benzyloxy-)

Figure 1 Structures of 7-substituted quinoline substrates and 7-hydroxyquinoline (7-quinolinol).

effect of smoking on 7-alkoxyquinoline metabolism was investigated using placentas from smoking and non-smoking mothers.

Methods

Chemicals

A series of 7-alkoxyquinolines (Figure 1) (7-methoxy- to 7-hexoxy- and 7-benzyloxyquinoline) and 7-quinolinol were synthesised as described by Mayer *et al.* (1989, 1990). All other chemicals were of the highest commercial grade.

Antibodies

Monoclonal antibodies were raised against purified rat liver P450s after treatment with 3-methylcholanthrene (Mab 1-7-1) (Park *et al.*, 1982), phenobarbitone (Mab 2-66-3) (Park *et al.*, 1984) and ethanol (Mab 1-91-3) (Ko *et al.*, 1987). Mab 1-68-11 was raised against purified rat liver cytochrome P450 2c/RLM5 (Park *et al.*, 1989). Mab HyHel-9 does not react with P450s and was used as a control.

Previous studies showed that Mab 1-7-1 inhibits the metabolism of xenobiotics in human tissues. Thus, hepatic 7-ethoxyresorufin *O*-deethylase activity was inhibited by 0 to 65% and placental 7-ethoxyresorufin *O*-deethylation and aryl hydrocarbon hydroxylase activities were inhibited by about 80% (Pelkonen *et al.*, 1986).

Tissue preparations

Human placenta samples Placentas were obtained from the Department of Gynaecology and Obstetrics of Oulu University Central Hospital, two of them (91/3 and 91/6) after normal vaginal delivery at term, one (91/8) after normal vaginal delivery a month before term and one (91/2) after Caesarean section at term. Placenta 91/6 was from a mother who had smoked 20 cigarettes daily until 1 month before delivery at which time she stopped smoking. Placenta 91/8 was from a mother who had smoked 10 cigarettes daily throughout her pregnancy. Connective tissue and coagulated blood was excised from trophoblastic tissue. The placenta was minced with scissors and homogenized with a knife homogenizer in four volumes of 0.1 M sodium-potassium phosphate buffer (pH 7.4) containing 20% v/v glycerol. Homogenates were centrifuged at 800 *g* for 20 min and the supernatant was centrifuged at 10,000 *g* for 30 min. The supernatant obtained after this second centrifugation was centrifuged at 100,000 *g* for 60 min and the microsomal pellet was stored at −70° C until used.

Human liver samples Two livers were from organ donors who had had fatal accidents. The other five samples were biopsies taken during laparotomy from patients with liver disease. In the histological examination of liver samples minor damage was noted but no severe damage or autolysis was present. Four of the donors were non-smokers, two smoked occasionally and the smoking status of one donor is unknown. Five donors consumed alcohol occasionally, one was a heavy drinker and the consumption of one donor is unknown. No correlation was observed between *O*-dealkylation activity and smoking or alcohol consumption. Three donors had not received any drug therapy. The liver that produced the highest activity was from a donor who had received oestrone and oestradiol therapy for 13 years. The therapy was stopped 2 years before biopsy. Three donors had received various drugs for different periods of time, but these treatments were not correlated to *O*-dealkylation activity. Liver samples were either processed within 1 h of removal and the microsomes stored at −70° C or they were first frozen in liquid nitrogen and processed later. Tissue from organ donors was removed within 30 min of death. The microsomal fractions were prepared by standard differential centrifugation as described by Raunio *et al.* (1990). Use of the human tissues was approved by the Ethics Committee of the Faculty of Medicine, University of Oulu.

Mouse and rat liver samples Six male Wistar rats and five female CD2F1 mice were used. Treatment of the animals was as described by Raunio *et al.* (1988). The microsomal fractions were prepared as described for human liver microsomes. Aliquots of the individual samples were pooled. Protein contents of all microsomal preparations were measured according to Bradford (1976).

Enzyme assays

7-Alkoxyquinoline *O*-dealkylation was measured by the method of Aitio (1978) with modifications. The reaction mixure was preincubated for 2 min at 37° C before adding 30 µl of 2 mM 7-alkoxyquinoline. The 7-alkoxyquinolines were dissolved in water and dimethyl sulphoxide (DMSO). The small amounts of DMSO used had no effect on enzyme activity. The final volume of the incubation solution was 0.5 ml and the amount of microsomal protein was 0.1 mg (liver) or 1 mg (placenta). The reaction was stopped by the addition of 0.5 ml 6% w/v trichloroacetic acid after 10 min incubation at 37° C. The reactions were linear with time and protein concentration. The samples were centrifuged for 10 min at 3000 rev min^{-1}. The supernatant (0.5 ml) was taken and 2 ml 1.6 M glycine-NaOH (pH 10.4) was added near to spectrofluorometer measurement. The excitation and emission wavelengths were 370 nm and 500 nm, respectively. A known concentration of 7-quinolinol was used as an

external standard in each series of measurements. The results of the enzyme assays are expressed as nmol or pmol 7-quinolinol produced per mg microsomal protein min^{-1}.

Immunoinhibition assays

Immunoinhibition assays were performed by adding the antibody to the incubation mixture 2 min before the reaction was started. In assays using liver tissue the amount of antibody protein was equal to the amount of microsomal protein. With placental preparations the amount of antibody protein was 5% of the amount of microsomal protein. All enzyme and immunoinhibition assays were carried out in duplicate.

Results

7-Alkoxyquinoline O-dealkylation by human liver microsomes

Seven human livers were used and all showed similar metabolic profiles. However, there were greater differences in activity between different liver preparations with substrates that were more rapidly metabolised.

Figure 2 shows mean values of the rate of product appearance from each substrate. The most rapidly metabolised 7-alkoxyquinoline derivative was 7-methoxyquinoline. Metabolic activity decreased as the length of the sidechain increased. The activity of 7-ethoxyquinoline was 45% of that of 7-methoxyquinoline and that of 7-hexoxyquinoline was only 7.5% of 7-methoxyquinoline activity. *O*-Debenzylation activity was 50% of *O*-demethylation activity.

Immunoinhibition of human liver enzyme activity

Inhibition of the metabolism of the five most rapidly metabolised 7-alkoxyquinoline derivatives was studied. The results are summarized in Table 1.

7-Alkoxyquinoline O-dealkylation by human placental microsomes

Four placentas were selected to investigate the influence of maternal cigarette smoking on 7-alkoxyquinoline metabolism. Two (91/2, 91/3) were from non-smoking and two (91/6, 91/8) were from smoking mothers. 7-Alkoxyquinoline metabolism by placentas from non-smoking mothers was consistently low for all substrates (Figure 3). The placentas from smokers metabolised 7-alkoxyquinolines considerably faster than non-induced placentas (Figure 3). 7-Alkoxyquinoline *O*-dealkylation activity increased as the length of the sidechain increased from one to four carbon atoms and decreased as it increased from four to six carbon atoms (Figure 2). The activity for 7-benzyloxyquinoline was similar to that for 7-butoxyquinoline. The greatest metabolism was seen with 7-benzyloxyquinoline by placenta 91/8; 59 times higher

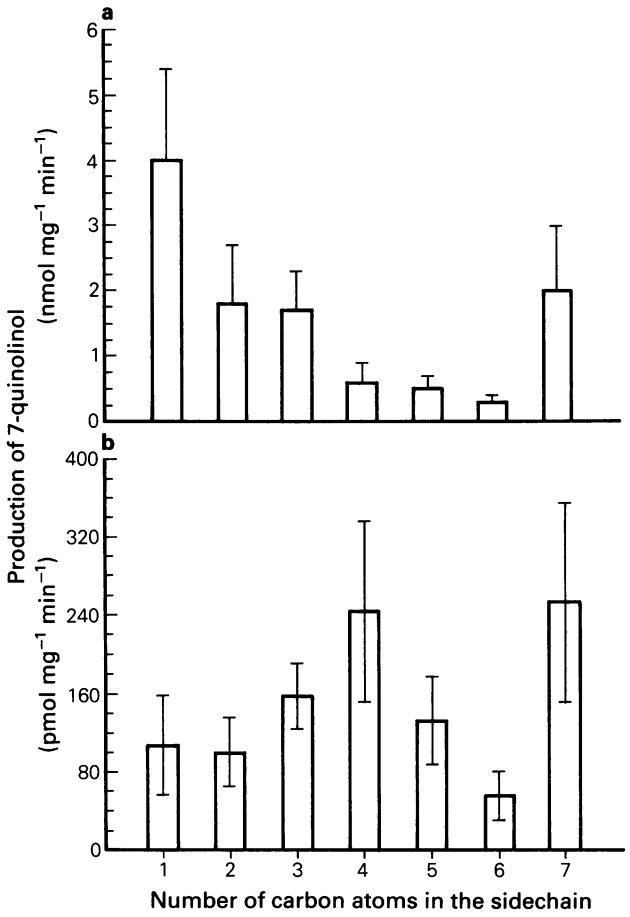


Figure 2 7-Alkoxyquinoline *O*-dealkylation (production of 7-quinolinol) by microsomes from a) human liver and b) smoking-induced placenta. The liver activities (means ± s.d.) are based on measurements using seven different liver samples. The placental activities (means and range) are based on measurements using two placentas from smoking mothers. The numbers below the histograms indicate the length of the sidechain. Number 7 indicates 7-benzyloxyquinoline. Note the different units of the y-axes.

Table 1 The effect of cytochrome P450 antibodies on 7-alkoxyquinoline *O*-dealkylation by microsomes of human liver. Values are based on duplicate measurements using one liver

	Enzyme activity (% of control)				
Antibody	*7-methoxyquinoline*	*7-ethoxyquinoline*	*7-propoxyquinoline*	*7-butoxyquinoline*	*7-benzyloxyquinoline*
None	100 (4.2)*	100 (3.0)	100 (2.0)	100 (0.9)	100 (1.4)
Mab 1-91-3	79	110	115	122	64
Mab 1-68-11	88	97	80	78	86
Mab 1-7-1	95	80	90	89	136
Mab 2-66-3	121	100	95	133	107

*Numbers in brackets refer to measured activity (nmol 7-quinolinol produced per mg protein min^{-1}).

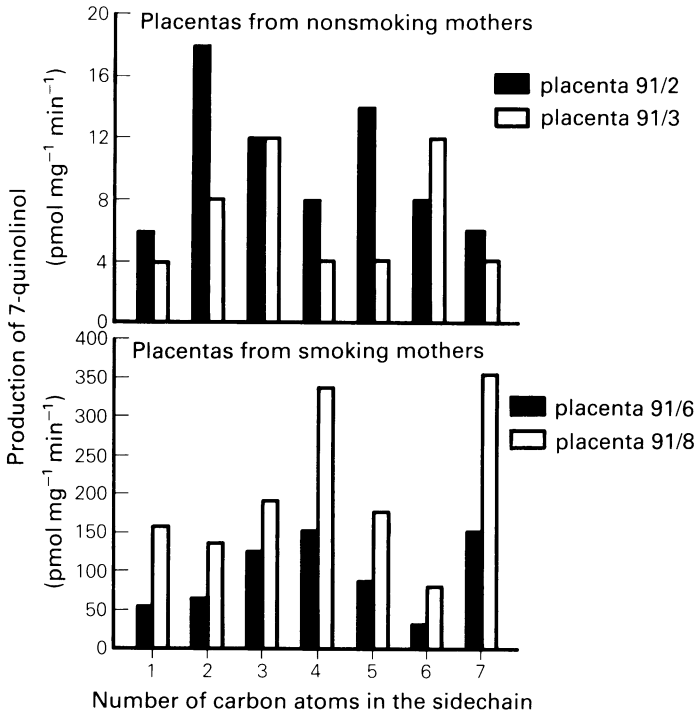


Figure 3 7-Alkoxyquinoline *O*-dealkylation (production of 7-quinolinol) by microsomes from four human placentas (duplicate determinations). The numbers below the histograms indicate the length of the sidechain. Number 7 indicates 7-benzyloxyquinoline.

than placenta 91/2. 7-Alkoxyquinoline metabolism by placenta 91/8 was 2.2-fold higher compared with placenta 91/6 (Figure 3). Placenta 91/6 was from a mother who had stopped smoking from a month before delivery. The level of enzyme induction could therefore have decreased from the time when the mother was smoking. However, when she was smoking mother 91/6 smoked twice as many cigarettes per day than mother 91/8.

Inhibition of smoking-induced enzyme activities by antibodies

The metabolism of 7-alkoxyquinolines induced by maternal cigarette smoking was inhibited by Mab 1-7-1 raised against MC induced rat liver P450 (Figure 4). The influence of the antibody/microsomal protein ratio on inhibition was determined with placenta 91/6 and 7-benzyloxyquinoline. Thus, a 1/20 (5%) antibody/microsomal protein ratio produced maximal inhibition. Using placenta 91/8 inhibition ranged from 33% (7-butoxyquinoline) to 60% (7-ethoxyquinoline) and using placenta 91/6 it ranged from 45% (7-methoxyquinoline) to 75% (butoxyquinoline). The amount of antibody protein used in these assays was 5% of the amount of microsomal protein.

Mab 2-66-3, raised against a purified PB-induced form of cytochrome P450, and HyHel-9, used as a control had no effect on metabolism (Figure 4).

O-Debenzylation by rat and mouse liver microsomes

Because induction of 7-benzyloxyquinoline metabolism in human placenta by cigarette smoking was an unexpected finding, 7-benzyloxyquinoline metabolism was also measured with MC-induced and control rat and mouse liver microsomes. MC-microsomes metabolised 7-benzyloxyquinoline faster than control microsomes in both species. Microsomes from control animals produced 1.4 (rat) and 10.6 (mouse) nmol 7-quinolinol per mg protein min^{-1}. MC-induced liver microsomes produced 6.8 (rat) and 14.9 (mouse) nmol 7-quinolinol per mg protein min^{-1}. The MC/control ratio was 4.9 for the rat and 1.4 for the mouse.

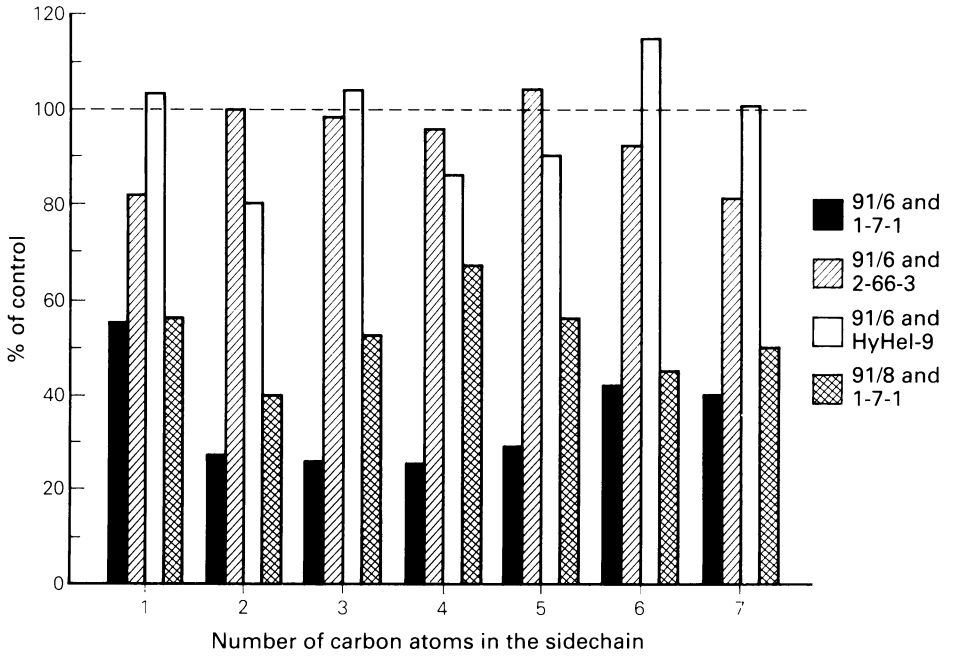


Figure 4 The effect of monoclonal antibodies (Mab) 1-7-1 (MC) and 2-66-3 (PB) on human placental 7-alkoxyquinoline *O*-dealkylation (duplicate determinations). *O*-dealkylation without any antibody was used as control (100%). Mab HyHel-9 does not react with cytochromes P450 and was used as unspecific control. The numbers below the histogram indicate the length of the sidechain. Number 7 indicates 7-benzyloxyquinoline.

Discussion

All of the 7-alkoxyquinolines were metabolised by human liver and placental microsomes. *O*-dealkylation by liver microsomes decreased as the length of the sidechain increased from one to six carbon atoms and *O*-debenzylation activity was slightly higher than *O*-deethylation activity. Non-induced placental microsomes had low metabolic activity towards all substrates. However, microsomes from placentas induced by smoking showed metabolic activity increasing from 7-methoxy- to 7-butoxyquinoline and decreasing from 7-butoxy- to 7-hexoxyquinoline. The metabolic activity towards 7-benzyloxyquinoline was similar to that for 7-butoxyquinoline.

7-Alkoxyquinoline *O*-dealkylase activity has been measured in rat (Mayer *et al.*, 1990) and mouse (Netter *et al.*, 1990; Hahnemann *et al.*, unpublished) livers. The activity in human liver was higher than in control rat liver but lower than in control mouse liver. The substrate profile of human liver differed considerably from that of rat liver profile but was similar to that of mouse liver. However, in the mouse 7-ethoxyquinoline was the most actively metabolised substrate while in the human 7-methoxyquinoline was metabolised more actively than 7-ethoxyquinoline. The substrate profile observed in placental tissue from smokers was closer to that in rat than mouse liver. The profile of metabolism in induced human placenta was very similar to that in control and PB-induced rat liver, excluding 7-benzyloxyquinoline and dissimilar to that in MC-induced rat liver.

In the present study we have shown that maternal cigarette smoking induces 7-alkoxyquinoline metabolism in placenta, being greatest to 7-butoxy- and 7-benzyloxyquinoline. The induction of 7-benzyloxyquinoline metabolism in placenta by maternal cigarette smoking was unexpected because previous work (Mayer *et al.*, 1990) with rat liver showed that MC induction decreased *O*-debenzylation. However, in the same study MC-induction resulted in a 50-fold lowering of the net K_m value, indicating an up-regulation of another enzyme (possibly CYP1A1 or CYP1A2). In the present study MC-induction caused a clear increase in *O*-debenzylation by rat liver and a smaller increase in mouse liver. The enzyme assay used in the present study differed from that used previously (Mayer *et al.*, 1990).

Unpublished studies by Hahnemann *et al.* with 7-ethoxyquinoline and mouse liver showed some inhibition with the polyclonal antibody anti-P450Coh. Our study with human liver and five 7-alkoxyquinoline derivatives indicated a 36% inhibition of *O*-debenzylation by Mab 1-91-3. However, as the *O*-debenzylation activity was fairly low and the activity varied between the replicates both in control and in inhibition assay, the inhibition by 1-91-3 could not be established with confidence.

Polycyclic aromatic hydrocarbons, present in cigarette smoke induce CYP1A1 in human placenta (Pasanen *et al.*, 1990; Sesardic *et al.*, 1990). The metabolic activities catalysed by CYP1A1 in placenta can be inhibited by Mab 1-7-1 raised against the MC-induced rat liver P450 isoenzyme (Fujino *et al.*, 1982, 1984; Pelkonen *et al.*, 1986). The results of the present study show that 7-alkoxyquinoline metabolism is also induced by maternal cigarette smoking and that this metabolic activity is partly inhibited by Mab 1-7-1. Thus, CYP1A1 appears to mediate 7-alkoxyquinoline metabolism in human placenta. However, Mab 1-7-1 does not inhibit *O*-dealkylation completely and the remaining activity appears to be higher than the basal activity of non-induced placentas. This suggests that another isoenzyme, which is also induced by cigarette smoking, participates in 7-alkoxyquinoline metabolism in the placenta. Alternatively, interaction between Mab 1-7-1 and human placental CYP1A1 may not lead to complete inhibition of catalytic activity. However, earlier studies have shown Mab 1-7-1 is able to inhibit smoking-induced placental 7-ethoxyresorufin *O*-deethylase and aryl hydrocarbon hydroxylase activities by 80–90% (Fujino *et al.*, 1984; Pelkonen *et al.*, 1986). Thus, incomplete inhibition is unlikely. It is also possible that the two smoking-induced placentas used in this study may have had considerably higher basal activities than the two placentas from non-smoking mothers studied and this would explain the non-1-7-1-inhibitable activity. Further studies with larger numbers of placenta samples are required to clarify the effects of smoking.

One of the reasons for developing the 7-alkoxyquinoline substrates was to determine whether the nitrogen atom in the aromatic ring affects substrate specificity with respect to P450 isoenzymes (Mayer *et al.*, 1989, 1990). The results of the present study suggest that none of the 7-alkoxyquinoline derivatives are P450-isoenzyme specific in human tissues indicating that the heterocyclic N atom has little influence.

This study was supported by Research Contract (no. 1051029) with The Academy of Finland and was carried out within the framework of the COST B1 action.

References

Aitio, A. (1978). A simple and sensitive assay of 7-hydroxycoumarin deethylation. *Anal. Biochem.*, **85**, 488–491.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

Burke, M. D. & Mayer, R. T. (1983). Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P-450-binding of phenoxazone and a homologous series of its *O*-alkylethers (alkoxyresorufins). *Chem. Biol. Interact.*, **45**, 243–258.

Fujino, T., Gottlieb, K., Manchester, D. K., Park, S. S., West, D., Gurtoo, H. L., Tarone, R. E. & Gelboin, H. V. (1984). Monoclonal antibody phenotyping of inter-individual differences in cytochrome P-450-dependent re-

actions of single and twin human placenta. *Cancer Res.*, **44**, 3916–3923.

Fujino, T., Park, S. S., West, D. & Gelboin, H. V. (1982). Phenotyping of cytochromes P-450 in human tissues with monoclonal antibodies. *Proc. Natl Acad. Sci. USA*, **79**, 3682–3686.

Guengerich, F. P. (1989). Characterization of human microsomal cytochrome P-450 enzymes. *Ann. Rev. Pharmac. Tox.*, **29**, 241–246.

Ko, I.-Y., Park, S. S., Song, B. J., Patten, C., Tan, Y., Hah, Y. C., Yang, C. S. & Gelboin, H. V. (1987). Monoclonal antibodies to ethanol-induced rat liver cytochrome P-450 that metabolizes aniline and nitrosamines. *Cancer Res.*, **47**, 3101–3109.

Lu, A. Y. H. & West, S. B. (1980). Multiplicity of mammalian microsomal cytochrome P-450. *Pharmac. Rev.*, **31**, 277–295.

Mayer, R. T., Netter, K. J., Heubel, F., Buchheister, A. & Burke, M. D. (1989). Fluorometric assay of hepatic microsomal monooxygenases by use of 7-methoxyquinoline. *Biochem. Pharmac.*, **38**, 1364–1368.

Mayer, R. T., Netter, K. J., Heubel, F., Hahnemann, B., Buchheister, A., Mayer, G. K. & Burke, M. D. (1990). 7-Alkoxyquinolines: New fluorescent substrates for cytochrome P450 monooxygenases. *Biochem. Pharmac.*, **40**, 1645–1655.

Nebert, D. W., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1987). The P450 superfamily: Recommended nomenclature. *DNA*, **6**, 1–11.

Nebert, D. W., Nelson, D. R., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1989). The P450 superfamily: Updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA*, **8**, 1–13.

Nebert, D. W., Nelson, D. R., Coon, M. R., Estabrook, R. W., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R. & Waxman, D. J. (1991). The P450 superfamily: Update on new sequences, gene mapping and recommended nomenclature. *DNA Cell Biol.*, **10**, 1–14.

Netter, K. J., Hahnemann, B., Mangoura, S. A., Feil, F., Tegtmeier, M., Mayer, R. T. & Legrum, W. (1990). Selective inducers of the Coh-locus enhance the metabolism of coumarin- and of quinoline-derivatives but not that of naphthalenes. *Adv. Exp. Med. Biol.*, **283**. In *Biological reactive intermediates IV*, eds Witmer, C. M., Snyder, R. R., Jollow, D. J., Kalf, J. J., Kocsis, J. J. & Sipes, I. G., pp. 353–357. New York: Plenum Press.

Park, S. S., Fujino, T., West, D., Guengerich, F. P. & Gelboin, H. V. (1982). Monoclonal antibodies that inhibit enzyme activity of 3-methylcholanthrene-induced cytochrome P-450. *Cancer Res.*, **42**, 1798–1808.

Park, S. S., Fujino, T., Miller, H., Guengerich, F. P. & Gelboin, H. V. (1984). Monoclonal antibodies to phenobarbital-induced rat liver cytochrome P-450. *Biochem. Pharmac.*, **33**, 2071–2081.

Park, S. S., Waxman, D. J., Lapenson, D. P., Schenkman, J. B. & Gelboin, H. V. (1989). Monoclonal antibodies to rat liver cytochrome P-450 2c/RLM5 that regiospecifically inhibit steroid metabolism. *Biochem. Pharmac.*, **38**, 3067–3074.

Pasanen, M., Haaparanta, T., Sundin, M., Sivonen, P., Vähäkangas, K., Raunio, H., Hines, R., Gustafsson, J.-Å. & Pelkonen, O. (1990). Immunochemical and molecular biological studies on human placental cigarette smoke-inducible cytochrome P-450-dependent monooxygenase activities. *Toxicology*, **62**, 175–187.

Pelkonen, O., Pasanen, M., Kuha, H., Gachalyi, B., Kairalumoa, M., Sotaniemi, E. A., Park, S. S., Friedman, F. K. & Gelboin, H. V. (1986). The effect of cigarette smoking on 7-ethoxyresorufin *O*-deethylase and other monooxygenase activities in human liver: analysis with monoclonal antibodies. *Br. J. clin. Pharmac.*, **22**, 125–134.

Raunio, H., Syngelmä, T., Pasanen, M., Juvonen, R., Honkakoski, P., Kairaluoma, M. A., Sotaniemi, E., Lang, M. A. & Pelkonen, O. (1988). Immunochemical and catalytical studies on hepatic coumarin 7-hydroxylase in man, rat, and mouse. *Biochem. Pharmac.*, **37**, 3889–3895.

Raunio, H., Valtonen, J., Honkakoski, P., Lang, M. A., Ståhlberg, M., Kairaluoma, M. A., Rautio, A., Pasanen, M. & Pelkonen, O. (1990). Immunochemical detection of human liver cytochrome P450 forms related to phenobarbital-inducible forms in the mouse. *Biochem. Pharmac.*, **40**, 2503–2509.

Sesardic, D., Pasanen, M., Pelkonen, O. & Boobis, A. R. (1990). Differential expression and regulation of members of the cytochrome P450IA gene subfamily in human tissues. *Carcinogenesis*, **11**, 1183–1188.

(Received 25 October 1991, accepted 21 April 1992)