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Cytochrome P4501A(CYP1A) induction in rat and man by the benzodioxino derivative, fluparoxan

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1. Fluparoxan is an α_2-adrenoceptor antagonist that has a relatively planar, tricyclic structure and was considered a potential substrate and inducer of cytochrome P4501A (CYP1A) enzymes.

2. Structure–activity analysis indicated some potential for CYP1A interaction, although its greater log *P* and molecular depth, compared with many CYP1A inducers, suggested fluparoxan would be a weak ligand for the aryl hydrocarbon (*Ah*) receptor and only a weak inducer.

3. *In vitro*, fluparoxan showed little affinity for the CYP1A enzymes. The compound was not metabolized by human CYP1A1 or 1A2 heterologously expressed in yeast and its rate of metabolism in rat and human microsomes was unaffected by the addition of the 1A inhibitor α-naphthoflavone. Furthermore, K_i's for fluparoxan against EROD activity were $>$ 4000-fold higher than those of α-naphthoflavone.

4. *In vivo*, however, fluparoxan did show some capacity for CYP1A induction. In rat, hepatic EROD activity increased approximately 40-fold with seven once-daily oral doses of fluparoxan (50 mg/kg, solution), and immunoblotting studies confirmed induction of CYP1A2, though not of 1A1. In man, administration of 11 twice-daily oral doses of fluparoxan (8 mg tablet) produced some reduction in plasma levels of orally administered phenacetin and in the ratio of phenacetin *AUC*/urinary paracetamol, consistent with increased *O*-deethylation.

Introduction

In order to develop safe and effective medicines, an awareness of the potential interactions between a new drug substance and metabolic enzymes is extremely important. The inhibition or induction of enzymes following drug administration can affect the circulating levels of endogenous compounds and dietary constituents as well as other xenobiotics; with potentially calamitous results (Tarrús *et al*. 1987a). Of particular importance in this area is the cytochrome P450 superfamily of haem-thiolate proteins (Parke 1990), which represents a multiplicity of both constitutive and inducible enzymes, capable of metabolising a broad range of substrates (Nebert and Gonzalez 1987).

Historically, our understanding of P450 induction (Okey 1990) has largely developed through studies on carcinogenic polycyclic aromatic hydrocarbons (PAHs) such as benzo(*a*)pyrene and 3-methylcholanthrene (3-MC) and aromatic amines such as 2-acetylaminofluorene, with the major PAH-induced cytochromes having been established as the P4501A (CYP1A) subfamily (Nebert *et al*. 1989). This subfamily (CYP1A) consists of two members, 1A1 and 1A2, (Nelson *et al*. 1996)

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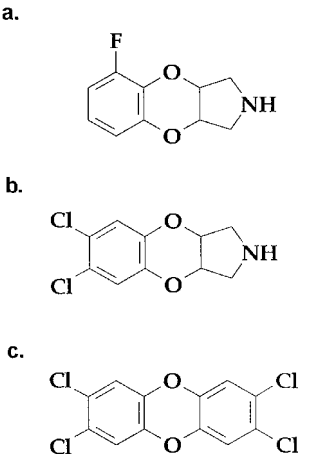

Figure 1. Comparative structures of benzodioxino derivatives and the potent CYP1A inducer, 2, 3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). (a) Fluparoxan (GR50360), (b) GR37459, (c) TCDD.

having approximately 70% nucleotide and amino acid sequence homologies (Kimura *et al*. 1984, Nelson and Strobel 1987, Fujii-Kuriyama *et al*. 1992) and both are inducible *via* the *Ah* receptor (Nebert *et al*. 1993, Hankinson 1995).

Whilst there is not necessarily a direct correlation between the potency of a polycyclic hydrocarbon as a CYP1A inducer and its carcinogenicity (Conney 1967), it is clear that many of the features which confer *Ah* receptor affinity upon a molecule are also those which render them potential mutagens/carcinogens (Ioannides and Parke 1993). Consequently, computational studies designed to try and predict the toxicity of such compounds are equally predictive of their *Ah* receptor binding affinity (Ioannides *et al*. 1994, Lewis *et al*. 1994). Extensive molecular modelling studies on a series of dioxins have shown that their potential *Ah* receptor binding capability can be predicted (QSAR correlations up to $r = 0{\cdot}95$) using molecular orbital calculated electronic parameters (e.g. frontier orbital energies) combined with a number of structural descriptors such as log *P*, area/depth^2 and length/width ratios (Lewis 1996).

Fluparoxan

Fluparoxan, 5-fluoro-2,3,3a,9a-tetrahydro-1*H*-[1,4]-benzodioxino-[2,3-c] pyrrole (figure 1a), is a potent α_2-adrenoceptor antagonist (Halliday *et al*. 1988), which has been shown to inhibit the central α_2-adrenoceptors in man (Johnson *et al*. 1995) and was developed as a potential anti-depressant. The compound progressed satisfactorily through *in vitro* mutagenicity testing and animal toxicity testing to clinical trials in man by 1988 (Gristwood 1990). However, development was halted in 1994 when the compound failed to show a clear clinical advantage over existing therapies.

Metabolic and pharmacokinetic studies (unpublished data) showed fluparoxan to be well absorbed following oral dosing in all species tested. Clearance was largely metabolic, with both oral and intravenous doses being excreted mainly *via* the urine ($> 90\%$ of administered dose), chiefly as phase II metabolites (sulphamic acid and carbamoyl glucuronide conjugates). The compound was well tolerated on repeat

oral administration to rat ($\leqslant$ 200 mg/kg/day) and dog ($\leqslant$ 80 mg/kg/day) for 12 months, although there was some reduction in weight gain at high doses and some evidence of increased liver weight in rat, but not in dog.

As an increase in liver weight could be indicative of changes in metabolic enzymes, investigations were carried out to assess the potential of fluparoxan to induce cytochromes P450, and particularly, in view of the relatively planar nature of the molecule, those of the CYP1A family. Indeed, a degree of structural similarity to the dioxins is apparent if the dichloro analogue of fluparoxan, GR37459 (figure 1b) is compared with the potent CYP1A inhibitor 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD ; figure 1c). This paper describes the calculation of the *Ah* receptor binding potential and CYP1A induction potential of fluparoxan and a series of *in vitro* interaction studies performed using rat and human liver microsomes, and human CYP1A isozymes heterologously expressed in yeast. Also described are *in vivo* studies carried out to monitor possible CYP1A induction by fluparoxan in rat, by measurement of microsomal ethoxyresorufin-*O*-deethylation (EROD) and immunoblotting, and a subsequent investigation in man, using phenacetin as the probe substrate (Conney *et al*. 1976, Pantuck *et al*. 1979).

Materials and methods

Reagents

Fluparoxan (GR50360) and its dichloro analogue, GR37459 (6,7-dichloro-2,3,3a,9a-tetrahydro-1*H*-[1,4]-benzodioxine-[2,3-c] pyrrole), were synthesized (as their hydrochloride salts) at Glaxo Research and Development (Greenford, UK). Phosphate-buffered saline (PBS, 100 mM, pH 7·4), NADPH (chemically reduced) and type H-2 glucuronidase/sulphatase (crude solution from *Helix pomatia*) were obtained from Sigma Chemicals (Poole, UK). Coomassie protein assay reagent was obtained from Pierce and Warriner (Chester, UK). Dimethyl sulphoxide (DMSO) and all other solvents and reagents used were Analar grade or equivalent.

Computational analysis

The potential *Ah* receptor binding capabilities (PEC_{50}) of fluparoxan and GR37459 were calculated from a previously derived (Lewis 1996) quantitative structure–activity relationship (QSAR) based on a series of structurally related 2,3-dichlorodibenzo-*p*-dioxins (DCDDs), and compared with TCDD. The PEC_{50} equates to the negative logarith of the drug concentration required to produce 50% displacement of TCDD from the *Ah* receptor. Sybyl software (Tripos Associates, St Louis, MO, USA) was used for the generation of molecular orbital-calculated electronic structural descriptors, and HazardExpert (Compudrug, Budapest, Hungary) for calculation of log *P* *via* the method of Rekker *et al*. (1993).

A QSAR expression was also derived for CYP1A induction potential, i.e. the anticipated fold increase in CYP1A activity per unit dose:

$$\log \text{CYP1A induction} = 2{\cdot}67 - 0{\cdot}15\ CR - 0{\cdot}40\ l/w + 0{\cdot}23 \log P,$$

where CR is the COMPACT radius = $\sqrt{(a/d^2 - 15)^2 + (\Delta E - 7)^2}$, l/w (length/width) = ratio of molecular length and width for the minimum energy geometries, a/d^2 (area/depth^2) = ratio of molecular area and square of molecular depth for the minimum energy geometries, and ΔE = energy difference between highest occupied and lowest unoccupied molecular orbitals. This relationship gives a 0·99 correlation with measured CYP1A induction (increased metabolic activity normalized for mmol/kg dose) for a series of diverse chemicals using the data reported by Ioannides and Parke (1993).

Microsomal metabolism and inhibition studies

In vitro metabolism and inhibition studies were performed using both rat and human liver microsomes. The latter were produced from tissue acquired as surgical waste from donor livers prepared for paediatric transplant and were considered essentially normal. Samples from one male (H24, age 16, previous treatment with antibiotics, cause of death meningitis) and one female donor (H26, age 18, previous treatment with antibiotics, dopamine and noradrenalin, cause of death subarachnoid haemorrhage) were used. Tissue homogenates were centrifuged at 9000 *g* to remove cell debris and the supernatants further centrifuged (100 000 *g*) to produce microsomal pellets, which were resuspended in PBS containing 20% (v/v) glycerol. In addition, microsomes containing the individual human CYP1A1

and 1A2 isozymes, heterologously expressed in *Saccharomyces cerevisiae* (Beresford *et al.* 1996), were obtained by courtesy of the University of Sheffield, Department of Medicine and Pharmacology. Microsomal protein contents were determined using Coomassie reagent (Bradford 1976) as directed by the suppliers and cytochrome P450 content was determined by CO difference spectroscopy (Omura and Sato 1964).

Microsomal incubations ($\leq$ 10 mg protein in PBS) were performed, in duplicate, at 37 °C in 1 ml total volumes containing NADPH at an initial concentration of 1 mM. Metabolism of fluparoxan (initial concentrations 15 or 50 μM), was monitored using tritium-labelled drug (prepared as a 1 mM solution in PBS). Aliquots of incubate were mixed with equal volumes of 0·4 M perchloric acid solution, centrifuged to remove precipitated protein and the supernatant analysed by hplc using a radiochemical detector (Berthold LB506). The effect of α-naphthoflavone (ANF), a potent inhibitor of CYP1A activity (Diamond and Gelboin 1969), on fluparoxan metabolism was determined following pre-incubation (2 min) with the inhibitor (10 μl in DMSO) prior to addition of the substrate.

For comparative purposes, microsomal ethoxyresorufin-*O*-deethylation (EROD) activity and the effect of ANF on this activity were similarly determined for an initial substrate (7-ethoxyresorufin) concentration of 15 μM (5 μl in DMSO). EROD measurements were based on the method of Burke and Mayer (1974), with detection of the fluorescent product (resorufin) at excitation and emission wavelengths of 522 and 588 nm respectively.

EROD activities were also measured using 7-ethoxyresorufin at an initial concentration of 5 μM. For determination of K_i's, fluparoxan and ANF were added in 5–10 μl DMSO and pre-incubated for 2 min prior to addition of substrate.

Induction studies in rat

Male, random-bred, Sprague-Dawley hooded rats, age 12 weeks, weight range 240–280 g, were supplied by Glaxo Research and Development Animal Breeding Unit and oral doses of fluparoxan were administered by gavage as solutions (5 ml/kg) in distilled water.

Animals were given seven once-daily oral doses of fluparoxan (0·1, 5·0 or 50 mg free base/kg) or water only and sacrificed by cervical dislocation, approximately 24 h after the final dose, for production of microsomes.

Microsomal EROD was determined as a measure of CYP1A (PAH-inducible) activity and, for comparison, aminopyrine *N*-demethylation (APND), as a traditional measure of phenobarbitone-inducible activity (CYP2B), was measured by trapping liberated formaldehyde with semicarbazide and measuring the resultant semicarbazone colourimetrically at 415 nm (Nash 1953).

In order to provide an estimate of bioavailability in these experiments, the fluparoxan plasma concentrations following 50 mg/kg oral doses were compared with those from an earlier pharmacokinetic experiment, carried out in support of toxicology studies, in which male rats were given single intravenous doses of fluparoxan at 5 mg/kg in physiological saline (2 ml/kg). Blood samples were obtained by vena caval exsanguination (three animals per time-point) under ether anaesthesia, centrifuged to yield plasma and the drug concentrations determined by hplc with UV detection (Beresford *et al.* 1992).

Immunoblotting. Microsomal proteins (50 μg) from the livers of vehicle and fluparoxan treated rats were separated on 8·5% SDS-polyacrylamide gels (1·5-mm thick) using the method of Laemmli (1970). Following Western blotting of the separated proteins onto nitrocellulose filters (Model TE22 Transfer Unit, Hoefer Scientific Instruments, Newcastle, UK) the CYP1A proteins were incubated with specific rat 1A1 (Sesardic *et al.* 1990b) and 1A2 (Edwards *et al.* 1993) rabbit antibodies, kindly supplied by the Royal Postgraduate Medical School, London. Visualization was achieved following incubation with alkaline phosphatase-linked goat anti-rabbit second antibody and reaction with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

Comparisons were made with hepatic microsomes from additional rats pretreated with intraperitoneal doses of either β-naphthoflavone (80 mg/kg/day for 4 days), isosafrole (150 mg/kg/day for 4 days) or 3-MC (80 mg/kg single dose) in corn oil.

Induction study in man

The effects of fluparoxan on the pharmacokinetics of phenacetin were monitored in 24 healthy male volunteers (aged 18–40 years) using a placebo-controlled, double-blind, cross-over study. The study was approved by the Ethical Review Committee of Glaxo Group Research and of Northwick Park Hospital, and was conducted in accordance with the provisions of the Declaration of Helsinki and revisions. Subjects received a full explanation of the nature and purposes of the investigation and provided their written informed consent for participation in the study.

Following a run-in period of 21 days, during which time they were not permitted caffeine-containing beverages or barbecued food, each volunteer received 11 oral doses of either fluparoxan (8 mg tablet) or placebo, twice-daily. Approximately 24 h before dose 1 and 24 h after dose 11 of each treatment period, a single oral dose of phenacetin was administered (900 mg suspended in 50 g hydroxypropylmethylcellulose with water to a total volume of 250 ml).

Blood samples were taken and plasma separated for phenacetin analysis. The compound was extracted from plasma using cyanopropyl solid phase extraction cartridges and quantified by hplc using a Spherisorb C18 column (100 × 4·6 mm), with a mobile phase of potassium dihydrogen orthophosphate (0·1 M)–methanol (33 : 67 v/v), at a flow rate of 1 ml/min and detection by UV absorption at 254 nm.

Urine samples were collected for determination of total paracetamol content following hydrolysis of possible conjugates with sulphatase and glucuronidase (2 h at 37 °C in 0·1 M, pH 5 acetate buffer). The hydrolysates were analysed by hplc using a Nucleosil C18 column (150 × 4·6 mm) and a mobile phase of phosphate buffer (0·5 M, pH 3·8)–methanol–ethyl acetate (1400 : 220 : 4 by vol.), running at 2 ml/min, with detection pf paracetamol by UV absorption at 242 nm.

Results

Potential for CYP1A induction by fluparoxan

As shown in table 1, using a QSAR expression derived for a series of structurally-related DCDDs, the potential *Ah* receptor binding affinities (PEC_{50}), calculated for fluparoxan (3·459) and even for the dichloro analogue, GR37459 (4·293), are significantly lower than that of TCDD (7·679). Using the QSAR expression for CYP1A induction capability, derived using a structurally diverse series of chemicals, the low values (log scale) for the benzodioxino compounds (0·456 and 0·684), emphasize their low induction potential compared with TCDD (2·503).

Microsomal metabolism of fluparoxan

Fluparoxan was readily metabolized in rat liver microsomes (720 pmol/min/mg protein in samples from control animals). The metabolism was NADPH-dependent but largely unaffected by the addition of ANF (figure 2). Under the same initial conditions (15 μM substrate, 50 μM inhibitor), in the same microsomes, ANF reduced EROD activity by 81% (from 91 to 17 pmol/min/mg protein). As shown in table 2, the rate of fluparoxan metabolism in different batches of rat microsomes was not reflected by the EROD activity present : pretreatment of rats with BNF producing a 350-fold increase in EROD, but no increase in fluparoxan turnover. Hence, fluparoxan does not appear to be a substrate for CYP1A isozymes in the rat.

Likewise, the compound did not appear to be a substrate for human CYP1A isozymes ; showing no detectable metabolism with either CYP1A1 (10 pmol) or 1A2 (20 pmol) heterologously expressed in yeast, despite good turnover (> 200 pmol/min/mg protein) in human liver microsomes (table 2). As with rat microsomes, the addition of ANF at a concentration (10 μM), which reduced EROD activity by almost 90% (from 28 to 3·3 pmol/min/mg protein), did not affect the metabolism of fluparoxan by human liver microsomes.

Determination of K_i's against EROD activity (figure 3) showed that fluparoxan has a low affinity for CYP1A, with values of 356 and 311 μM being obtained in expressed human CYP1A1 and human liver microsomes respectively, compared with 23 and 72 nM for ANF in the same samples, i.e. at least a 4000-fold difference.

Induction of CYP1A in rat by fluparoxan

Measurement of plasma fluparoxan levels confirmed good oral absorption in the rat, with the *AUC* for a single 50 mg/kg dose (458 μg/ml h) being slightly in excess of the dose-corrected *AUC* (449 μg/ml h) obtained for a single intravenous dose (5 mg/kg).

The administration of seven, once-daily high oral doses (50 mg/kg) did not

Table 1. Comparison of physicochemical parameters used in assessing the potential *Ah* receptor binding affinity (PEC_{50}) and CYP1A induction potential of the benzodioxino derivatives, fluparoxan and GR37459, and the potent CYP1A inducer, 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD).

	Parameter						
Compound	log *P*	a/d^2	ΔE	l/w	COMPACT radius	PEC_{50}	CYP1A induction potential (log)
Fluparoxan	0·862	3·025	9·326	1·455	12·199	3·459	0·456
GR37459	2·125	3·166	8·884	1·693	11·983	4·293	0·684
TCDD	7·256	7·599	8·090	1·785	7·481	7·679	2·503

log *P* = calculated logarithm of the octanol/water partition coefficient; a/d^2 (area/depth^2) = ratio of molecular area and square of molecular depth for the minimum energy geometries; ΔE = energy difference between highest occupied and lowest unoccupied molecular orbitals; l/w (length/width) = ratio of molecular length and width for the minimum energy geometries; COMPACT radius = $\sqrt{(a/d^2-15)^2+(\Delta E-7)^2}$.

These parameters were used to calculate the potential *Ah* receptor binding affinities (PEC_{50}) and CYP1A induction potential based on QSAR expressions derived for a diverse series of possible CYP1A inducers (see Materials and methods for details). Both values are clearly lower for fluparoxan than TCDD, principally due to the much lower log *P* and reduced a/d^2 ratio of the former.

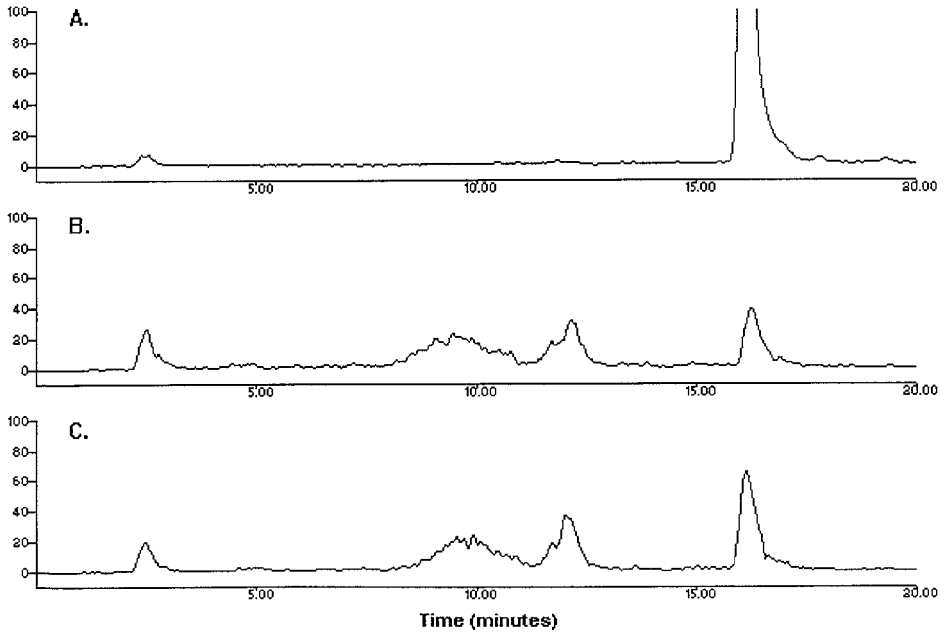

Figure 2. Metabolism of fluparoxan by rat liver microsomes. Tritium-labelled fluparoxan (15 μM) was incubated (37 °C) for 10 min with rat hepatic microsomes (5 mg protein) (A) in the absence or (B) in the presence of NADPH (1 mM) and (C) with NADPH and the CYP1A inhibitor α-napthoflavone (50 μM). Chromatograms illustrate the pattern of radioactivity obtained on hplc (*y*-axis units = counts s^{-1}) following precipitation of microsomal proteins with perchloric acid solution (0·4 M). Microsomal metabolism of fluparoxan (Rt ~ 16 min) is clearly NADPH dependent, but is largely unaffected by addition of the inhibitor α-naphthoflavone. Under the same initials conditions (15 μM substrate, 1 mM NADPH) in the same microsomes, 50 μM α-naphthoflavone reduced EROD activity by > 80% (data not shown).

Table 2. Comparative microsomal metabolic data for fluparoxan and 7-ethoxyresorufin.

Microsomes	Metabolic rate (/min/mg protein)	
	7-Ethoxyresorufin (pmol product formed)	Fluparoxan (pmol substrate metabolized)
Control rat	3·8 (±0·6)	720 (±143)
Bnf rat	1320 (±33)	80 (±15)
Iso rat	94 (±2)	227 (±41)
Flu rat	118 (±4)	507 (±76)
Human (male, H24)	21 (±1)	368 (±29)
Human (female, H26)	36 (±4)	806 (±96)
CYP1A1	37 (±3)	*n.d.
CYP1A2	1·4 (±0·3)	n.d.

* n.d. = < 15 pmol/min/mg protein.

The microsomal metabolism of 7-ethoxyresorufin (initial conc. 5 μM) was determined by spectrophotometric measurement of the fluorescent product (resorufin), and fluparoxan metabolism (initial conc. 50 μM) determined by radiochromatographic monitoring of tritiated parent compound. Metabolic rates were compared in hepatic microsomes from untreated, β-naphthoflavone (Bnf, 80 mg/kg/day × 4), isosafrole (Iso, 150 mg/kg/day × 4) or fluparoxan (Flu, 50 mg/kg/day × 7) treated rats (pooled samples from three animals), in samples of human liver microsomes (H24 and H26) and in microsomes containing human CYP1A1 (25 pmol/mg protein) or 1A2 (12 pmol/mg protein) isozymes heterologously expressed in yeast.

Data represent the mean of triplicate determinations (±SD) and show no obvious correlation between the rate of fluparoxan metabolism and that for the recognized CYP1A substrate 7-ethoxyresorufin.

produce any marked change in the plasma profile of the drug; with peak plasma concentrations of 59·6 and 57·0 μg/ml at 0·5 h post-dose and the calculated absorption and elimination half-lives being approximately 0·3 and 3·3 h respectively on both occasions. As shown in figure 4, this same oral dosage regimen did not produce any change in hepatic APND, but did result in a significant ($p < 0·01$) increase in EROD. An increase in EROD activity was also seen at the lower doses of 5·0 and 0·1 mg/kg, and it was subsequently found that a single dose of fluparoxan at 50 mg/kg produced a significant increase in EROD activity (data not shown).

Immunoblotting of microsomes from the fluparoxan treated rats showed a progressive increase in the level of CYP1A2 protein with dose (0·1, 5·0 and 50 mg/kg), but without increasing 1A1. This selectivity is illustrated in figure 5 by comparing high oral dose (50 mg/kg) fluparoxan microsomes with those from rats given intraperitoneal isosafrole, β-naphthoflavone or 3-methylcholanthrene. At the total protein loading used here (50 μg), there was no detectable CYP1A2 in microsomes from vehicle dosed rats, but the band intensity from fluparoxan dosed animals was similar to that from isosafrole treated animals, which also showed a similar level of EROD activity (around 100 pmol/min/mg). Similarly, CYP1A1 was undetectable in control microsomes, but, in contrast to 1A2, this remained undetectable after dosing with either fluparoxan or isosafrole. Based on the blotting of purified CYP1A proteins (data not shown), the limit of sensitivity for this procedure is considered to be about 0·25 pmol. Hence, neither compound had increased CYP1A1 concentrations > 5 pmol/mg microsomal protein. The CYP1A1 isozyme was, however, readily detectable in microsomes following BNF and 3-MC dosing; the EROD activity being considerably higher in these microsomes (> 1000 pmol/min/mg protein).

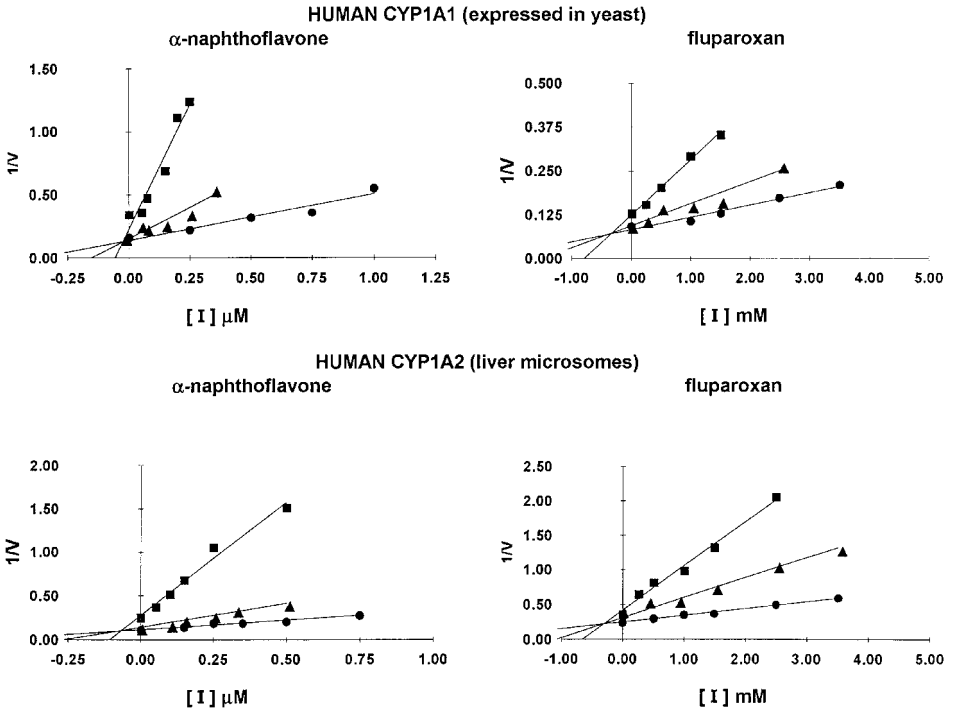

Figure 3. Effect of fluparoxan addition on microsomal ethoxyresorufin-*O*-deethylation (EROD). Microsomes from human liver (0·25 mg protein) or *Saccharomyces cerevisiae* cells heterologously expressing human CYP1A1 (10 pmol P450) were incubated with 7-ethoxyresorufin following pre-incubation (2 min at 37 °C) with fluparoxan and NADPH (1 mM), and the formation of resorufin (V = pmol/min) monitored over 5 min. The double reciprocal plots illustrate the determination of K_i using three ethoxyresorufin concentrations (■, 0·2; ▲, 1·0; ●, 5·0 μM) over a range of fluparoxan concentrations. Each point represents the mean of triplicate determinations. Comparative data are provided for the known CYP1A inhibitor α-naphthoflavone, in the same microsomes at the same three substrate concentrations, and show an approximately 4000-fold greater affinity for the enzymes than fluparoxan.

Induction of CYP1A in man by fluparoxan

Considerable variability in the plasma concentration/time profiles for phenacetin were seen between volunteers before and after both placebo and fluparoxan treatment, with four pretreatment and seven post-treatment sample sets containing no detectable drug (< 20 ng/ml plasma). A non-parametric statistical method, as described by Koch (1972), was therefore applied to the data. This indicated a statistically significant (p = 0·040) reduction in maximum phenacetin concentration following fluparoxan treatment (median 369 ng/ml) compared with placebo (median 456 ng/ml) and a reduction in the area under the phenacetin plasma concentration/time curve (from a median value of 441 to 407 h ng/ml) which approached statistical significance (p = 0·070). An illustration of these changes is provided by the plots of median plasma concentrations in figure 6. The suggestion that the changes were the result of increased phenacetin metabolism, rather than decreased absorption, was supported by a significant decrease (p = 0·025) in the ratio of phenacetin *AUC*/paracetamol excreted in the urine following fluparoxan treatment (from 2·9 to 1·9), compared with placebo (3·2 both before and after treatment).

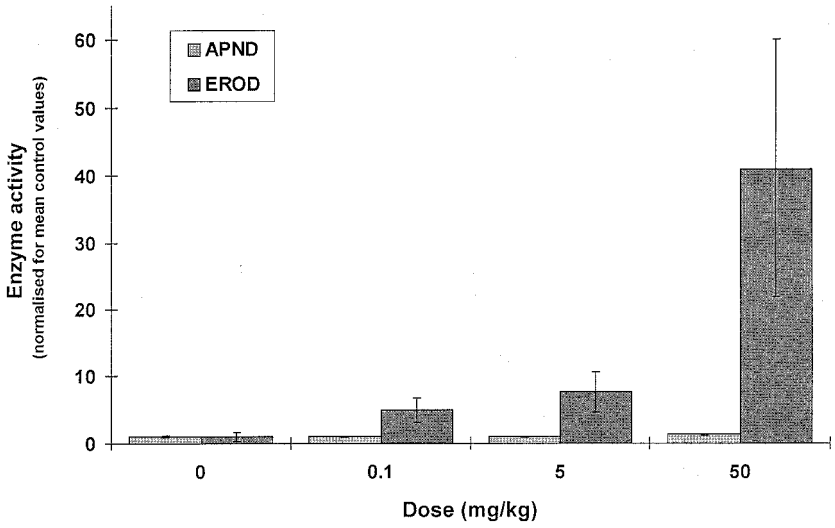

Figure 4. Effect of fluparoxan administration on hepatic aminopyridine-*N*-demethylation (APND) and ethoxyresorufin-*O*-deethylation (EROD) in the rat. Animals (n = 4 per treatment) were given seven once-daily oral doses of fluparoxan (0·1, 5·0 or 50 mg/kg) in water (or water only) and hepatic microsomes prepared approximately 24 h after the final dose. The histograms illustrate the change (±SD) in microsomal APND and EROD rates compared with the mean for vehicle-dosed controls and show little effect of the compound on APND but a significant induction of EROD.

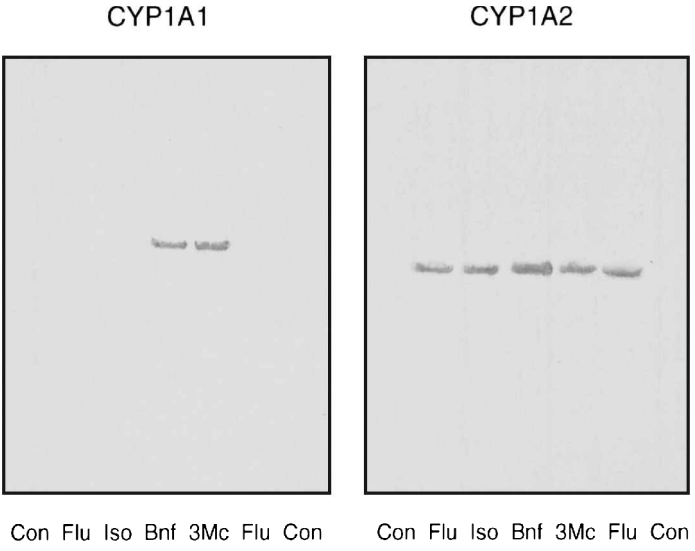

Figure 5. Induction of rat hepatic CYP1A proteins by fluparoxan. Hepatic microsomes were prepared from rats given seven once-daily oral doses of fluparoxan (0·1, 5·0 and 50 mg/kg) in water or water only. Microsomal proteins (50 μg samples) were separated on SDS- polyacrylamide gels (Laemmli 1970), transferred to nitrocellulose filters (Western blotting) and probed with either CYP1A1 or 1A2 specific antibodies. Samples from three rats at each dose level were probed individually and showed a progressive increase in CYP1A2 blot intensity with dose, but with CYP1A1 remaining undetectable (< 0·25 pmol). The immunoblot shown compares microsomes pooled from the 3 high oral dose (50 mg/kg) fluparoxan (Flu) rats with those given water only (Con) and animals given intraperitoneal doses of the known CYP1A inducers, isosafrole (Iso), β-naphthoflavone (Bnf) and 3-methylcholanthrene (3Mc).

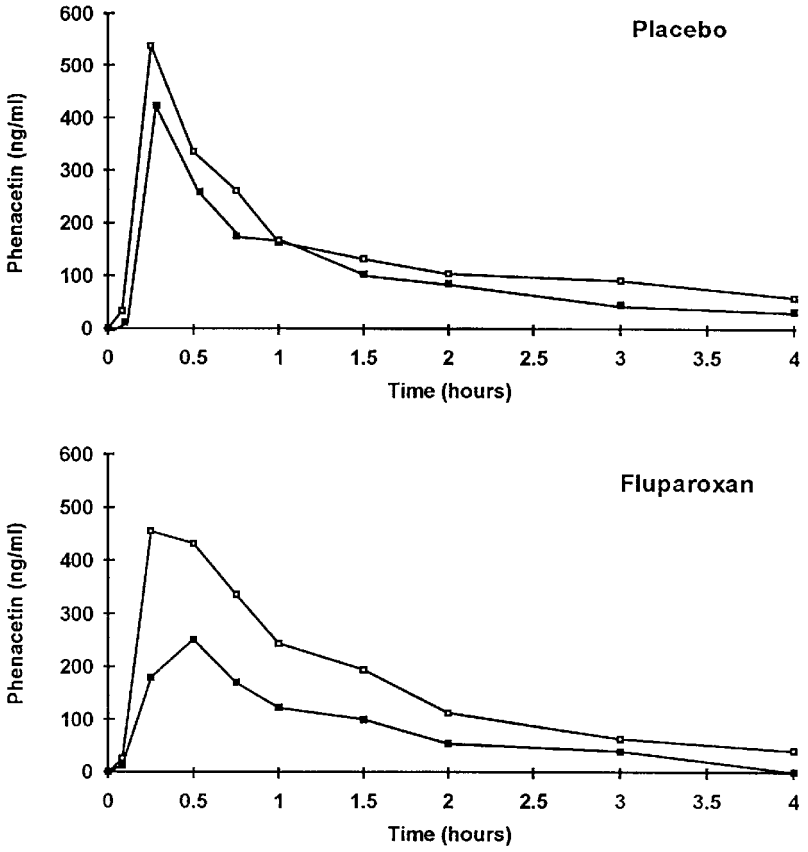

Figure 6. Effect of fluparoxan administration on plasma phenacetin concentrations in man. Male volunteers (n = 24) were given single oral doses of phenacetin (900 mg suspension) at each end of a treatment period comprising 11 oral doses of either fluparoxan (8 mg tablet) or placebo, twice-daily. The graphs represent the median phenacetin plasma concentrations from the 24 volunteers, determined before and after placebo or fluparoxan treatment. The plots are intended to illustrate the overall decrease in C_{max} (p = 0·040) and AUC (p = 0·070) apparent due to fluparoxan treatment and no attempt has been made to show the wide inter- and intra-subject variability seen before and after both treatments.

Discussion

The CYP1A enzymes have long been associated with the activation of chemical mutagens and carcinogens and, more recently, linked with other potentially procarcinogenic cellular events such as the activation of protein kinase *c* and phosphorylation of EGF (reviewed in Beresford 1993, Ioannides and Parke 1993). These effects have largely been attributable to highly lipophilic, planar, polycyclic aromatic compounds and aromatic amines, which are often substrates for the CYP1A subfamily as well as potent inducers of the enzymes *via* the *Ah* receptor. Induction *via* this receptor results in an increase in both CYP1A1 and 1A2 activity (Nebert and Jones 1989) and it has proved possible to correlate the *Ah* receptor binding affinity to the mutagenic/carcinogenic potential of such compounds (Ayrton *et al*. 1990, Lewis *et al*. 1994). This approach may be of considerable value in future if it would permit the calculation of potential mutagenicity before a compound has even been synthesized.

Fluparoxan is a relatively planar aromatic amine, and, although the compound

was not found to be a mutagen in cultured human peripheral lymphocytes and did not cause gene mutation when administered to Chinese hamster fibroblasts in culture (unpublished data), the potential *Ah* receptor binding affinity (PEC_{50}) and CYP1A induction capability were calculated. Using a QSAR expression generated for a series of DCDDs, the PEC_{50} computed for fluparoxan was extremely low compared to a wide range of known CYP1A inducers (Lewis 1996). Indeed, even the dichloro analogue of fluparoxan, GR37459 (figure 1b), which shows a considerable structural similarity to the highly potent CYP1A inducer, TCDD (figure 1c), shows a low PEC_{50} (table 1). Likewise, the CYP1A induction potential (log scale), calculated using a QSAR expression derived for a series of structurally diverse chemicals, was extremely low for fluparoxan and GR37459 (0·456 and 0·684 respectively), compared with TCDD (2·503) or, as previously calculated (Lewis 1996), to 3-MC (2·371) or BNF (2·929). The low calculated values for fluparoxan, in part, result from the relatively low log *P* and are reflected in the poor affinity for the CYP1A isozymes apparent in the *in vitro* studies (table 2, figure 3). Despite this, the data obtained *in vivo* in the rat (figure 4) demonstrate that administration of fluparoxan *can* produce hepatic CYP1A induction. If the assumption is made that induction was maximal after the first dose, then the actual increase in EROD activity (table 2), normalized for dose (mmol/kg), is much higher than anticipated for fluparoxan (119), but is about 10-fold lower than for BNF in this study (1180).

Interestingly, the greater molecular 'depth' (compared to the TCDDs) imparted to fluparoxan by the saturated pyrrole ring, has previously been noted as a possible descriptor for CYP1A2 versus 1A1 selectivity for aromatic amines (Lewis *et al.* 1994) and immunoblotting of the rat microsomes indicated that induction by fluparoxan is CYP1A2 selective (figure 5). This additional depth will also be a feature of the CYP1A2 selective methylenedioxyphenyl (MDP) compounds, isosafrole and piperonyl butoxide (Adams *et al.* 1994), and may indicate that the hydrocarbon-responsive element differs between the CYP1A1 and 1A2 genes: the form of the resultant complex between the *Ah* receptor and different ligands allowing differential induction. For example, the tight binding of TCDD may produce a conformational change compatible with both 1A1 and 1A2 genes, whilst a weak, deeper ligand may only permit attachment to the 1A2 gene. Such differentiation might also apply for induction through post-transcriptional stabilisation of mRNA, which has been shown to play a greater role in CYP1A2 induction than 1A1 induction (Pasco *et al.* 1988). In this respect, it is perhaps worth noting that preliminary data (not shown) on the dichloro analogue of fluparoxan, GR37459, suggests that it is capable of inducing both CYP1A1 and 1A2.

In addition to the MDPs a number of tricyclic compounds, such as acenaphthylene and dibenzofuran, have also been shown to act as CYP1A2-selective inducers. As this induction occurs in TCDD receptor-deficient mice, it has been proposed that the selectivity is *via* an *Ah* receptor-independent mechanism (Cook and Hodgson 1985, Chaloupka *et al.* 1994). In the case of the MDPs, induction is a two-phase process in which increased CYP1A2 protein synthesis follows an initial inhibition of the enzyme by formation of MDP metabolite-P450 complexes (Ryu *et al.* 1995), and a number of studies have suggested the presence of an autoregulatory loop in which the presence of functioning CYP1A protein negatively controls its own gene expression (Puga *et al.* 1990, RayChaudhuri *et al.* 1990, Jorgensen and Antrup 1996). This may involve some, as yet unidentified, endogenous substrate for the enzymes, the concentration of which would rise in the presence of inhibitors and

subsequently increase CYP1A gene transcription and protein production (Beresford 1993, Reick *et al.* 1994). With fluparoxan, although the *in vitro* metabolic data presented here (figures 2 and 3, table 2) show little evidence that the compound is either a substrate or inhibitor for the CYP1A enzymes, it is worth noting that after oral dosing at 50 mg/kg, the maximum plasma concentrations in rats (around 60 μg/ml or 380 μM) do exceed the K_i's calculated for both CYP1A1 (356 μM) and 1A2 (311 μM). Hence, it remains possible that CYP1A induction by fluparoxan could result from some negative feedback mechanism activated by a decrease in enzyme activity; though it is not clear why this should be CYP1A2 selective, unless the K_i against 1A1 in rat is higher than that for the human isozyme.

In man, hepatic CYP1A2 activity appears to vary substantially between individuals (Sesardic *et al.* 1988, Lucas *et al.* 1993), which makes the estimation of induction due to drug administration difficult. In addition to genotypic variability, phenotypic differences due to the effects of dietary and environmental chemicals on CYP1A2 (Conney *et al.* 1976, Pantuck *et al.* 1979) make it difficult to assess the clinical relevance of any induction seen. This has been particularly well illustrated by debates over the significance of CYP1A induction caused by the gastric acid suppressant, omeprazole (Petersen 1995). In the study performed herein, the exposure of volunteers to phenacetin before and after fluparoxan treatment was used as an indicator of CYP1A2 activity and showed a decrease in exposure (as plasma *AUC*) following drug treatment. Unfortunately, considerable variability in the plasma concentration/time profiles for phenacetin were seen between volunteers before and after both placebo and fluparoxan treatment. This probably reflects inherent variability in the first-pass metabolism of phenacetin, as well as differences in absorption rates, as demonstrated by Raaflaub and Dubach (1975). Whilst the clearance of phenacetin is not due solely to CYP1A2 activity (Mineshita *et al.* 1986), the enzyme is considered to be largely responsible for its *O*-deethylation to paracetamol (Sesardic *et al.* 1988). Consequently the plasma phenacetin *AUC* and the total urinary excretion of paracetamol were compared and the ratio of these parameters did indicate a significant increase in *O*-deethylation following fluparoxan treatment. However, in view of the variability seen between individuals and the known effects of diet and environment on CYP1A2 previously mentioned, as for omeprazole, the change was not considered to be of clinical concern.

Individual variability considered, CYP1A2 is the third major isoform present in human liver (Shimada *et al.* 1994). In untreated laboratory rats, however, the hepatic CYP1A2 content would appear to be negligible (Nedelcheva and Gut 1994). Certainly the level of EROD in the control rat microsomes prepared in the current work, was lower (at least 5-fold) than that of the human liver microsomes when expressed per mg microsomal protein (table 2), and this activity in the rat may mainly be due to CYP2D6 (Burke *et al.* 1994). The situation therefore exists that a potentially major route for metabolism of a xenobiotic in man may be only a minor route in the species most likely to be used for its metabolism and toxicity testing. This would be particularly important if the metabolic product was an activated chemical species.

Currently, inference of CYP1A involvement in metabolic pathways is made using microsomes from rats dosed with inducers such as 3-MC, BNF and isosafrole, in combination with selective CYP1A inhibitors such as ANF, furafylline (Sesardic *et al.* 1990) or anti-CYP1A antibodies (Edwards *et al.* 1993). Of the inhibitors, furafylline has proved to be the most selective CYP1A2 inhibitor and can be

employed *in vivo* (Tarrús *et al*. 1987b), although it exhibits a considerable interspecies difference in potency (Sesardic *et al*. 1990a). The inducers listed above are themselves potentially toxic, with only isosafrole being relatively selective for the induction of rat hepatic 1A2 (Thomas *et al*. 1983) and, unfortunately, known to bind to the enzyme which it induces (Ryan *et al*. 1980). Considerably more work would be required to confirm that fluparoxan has minimal effects on other metabolic enzymes (including phase II systems). However, its minimal interaction with the CYP1A2 isozyme which it induces could make the compound a useful tool for *in vitro* and *in vivo* studies on this isozyme or to investigate *Ah* receptor-independent mechanisms of CYP1A induction.

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References

Adams, N. H., Levi, P. E. and Hodgson, E., 1983, Regulation of cytochrome *P*-450 isozymes by methylenedioxyp henyl compounds. *Chemico-Biological Interactions*, **86**, 255–274.

Ayrton, D. A., McFarlane, M., Walker, R., Neville, S. and Ioannides, C., 1990, The induction of P450 I proteins by aromatic amines may be related to their carcinogenic potential. *Carcinogenesis*, **11**, 803–809.

Beresford, A. P., 1993, Cyp1A1: friend or foe? *Drug Metabolism Reviews*, **25**, 503–517.

Beresford, A. P., Caswell, K., Chambers, R. and Kirk, I. P., 1992, Advantages of achiral hplc as a preparative step for chiral analysis in biological samples and its use in toxicokinetic studies. *Xenobiotica*, **22**, 789–798.

Beresford, A. P., Taylor, R. J., Ashcroft, J.-A., Ayrton, J., Tucker, G. T. and Ellis, S. W., 1996, Expression of human cytochrome P450 1A1 (CYP1A1) in *Saccharomyces cerevisiae* inhibits cell division. *Xenobiotica*, **26**, 1013–1023.

Bradford, M. M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein–dye binding. *Annals of Biochemistry*, **72**, 248–254.

Burke, M. D. and Meyer, R. T., 1974, Ethoxyresorufin: direct fluorometric assay of a microsomal *o*-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metabolism and Disposition*, **2**, 583–588.

Burke, M. D., Thompson, S., Weaver, R. J., Wolf, C. R. and Mayer, R. T., 1994, Cytochrome P450 specificities of alkoxyresorufin *O*-dealkylation in human and rat liver. *Biochemical Pharmacology*, **48**, 923–936.

Chaloupka, K., Santostefano, M., Goldfarb, I. S., Liu, G., Myers, M. J., Tsyrolv, I. B., Gelboin, H. V., Krishnan, V. and Safe, S., 1994, Aryl hydrocarbon (Ah) receptor-independent induction of Cyp1a2 gene expression by acenaphthylene and related compounds in B6C3F1 mice. *Carcinogenesis*, **15**, 2835–2840.

Conney, A. H., 1967, Pharmacological implications of microsomal enzyme induction. *Pharmacological Reviews*, **19**, 317–366.

Conney, A. H., Pantuck, E. J., Hsiao, K.-C., Garland, W. A., Anderson, K. E., Alvares, A. P. and Kappas, A., 1976, Enhanced phenacetin metabolism in human subjects fed charcoal-broiled beef. *Clinical Pharmacology and Therapeutics*, **20**, 633–642.

COOK, J. C. and HODGSON, E., 1985, The induction of cytochrome *P*-450 by isosafrole and related methylenedioxyp henol compounds, *Chemico-Biological Interactions*, **54**, 299–315.

DIAMOND, L. and GELBOIN, H. V., 1969, Alpha-naphthoflavone: an inhibitor of hydrocarbon cytotoxicity and microsomal hydroxylase. *Science*, **166**, 1023–1025.

EDWARDS, R. J., MURRAY, B. P., SINGLETON, A. M., MURRAY, S., DAVIES, D. S. and BOOBIS, A. R., 1993, Identification of the epitope of an anti-peptide antibody which binds to CYP1A2 in many species including man. *Biochemical Pharmacology*, **46**, 213–220.

FUJII-KURIYAMA, Y., IMATAKA, H., SOGAWA, K., YASUMOTO, K.-I. and KIKUCHI, Y., 1992, Regulation of CYP1A1 expression. *FASEB Journal*, **6**, 706–710.

GRISTWOOD, W. E., 1990, Determination of fluparoxan (GR50360) in plasma by gas chromatography. *Journal of Chromatography*, **527**, 436–440.

HALLIDAY, C. A., JONES, B. J., STRAUGHAN, D. W., TYERS, M. B., WALSH, D. M., WALSH, H. and WISE, H., 1988, GR50360, a novel and highly selective α_2-adrenoceptor antagonist. *British Journal of Pharmacology*, **95**, 751P.

HANKINSON, O., 1995, The aryl hydrocarbon receptor complex. *Annual Review of Pharmacology and Toxicology*, **35**, 307–340.

IOANNIDES, C., LEWIS, D. F. V. and PARKE, D. V., 1994, The use of computers in the safety evaluation of drugs and other chemicals. *European Journal of Drug Metabolism and Pharmacokinetics*, **3**, 225–233.

IOANNIDES, C. and PARKE, D. V., 1993, Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis. *Drug Metabolism Reviews*, **25**, 485–501.

JOHNSON, M. A., BLACKWELL, C. P. and SMITH, J., 1995, Antagonism of the effects of clonidine by the α_2-adrenoceptor antagonist, fluparoxan. *British Journal of Clinical Pharmacology*, **36**, 477–483.

JORGENSEN, E. C. B. and AUTRUP, H., 1996, Autoregulation of human CYP1A1 gene promoter activity in HepG2 and MCF-7 cells. *Carcinogenesis*, **17**, 435–441.

KIMURA, S., GONZALEZ, F. J. and NEBERT, D. W., 1984, The murine Ah locus. Comparison of the complete cytochrome P1-450 and P3-450 cDNA nucleotide and amino acid sequences. *Journal of Biological Chemistry*, **259**, 10 705–10 713.

KOCH, G. G., 1972, The use of non-parametric methods in the statistical analysis of the two-period change-over design. *Biometrics*, **28**, 577–584.

LAEMMLI, U. K., 1970, Change of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

LEWIS, D. F. V., 1996, Quantitative structure-activity relationships in substrates, inducers and inhibitors of cytochrome P4501 (CYP1). *Drug Metabolism Reviews* (in press).

LEWIS, D. F. V., MOEREELS, H., LAKE, B. G., IOANNIDES, C. and PARKE, D. V., 1994, Molecular modeling of enzymes and receptors involved in carcinogenesis: QSARs and COMPACT-3D. *Drug Metabolism Reviews*, **26**, 261–285.

LUCAS, D., BERTHOU, F., DRÉANO, Y., LOZAC'H, P., VOLANT, A. and MÉNEZ, J.-F., 1993, Comparison of levels of cytochrome P-450, CYP1A2, CYP2E1, and their related monooxygenase activities in human surgical liver samples. *Alcoholism: Clinical and Experimental Research*, **17**, 900–905.

MINESHITA, S., EGGERS, R., KITTERINGHAM, N. R. and OHNHAUS, E. E., 1986, Determination of phenacetin and its major metabolites in human plasma and urine by high performance liquid chromatography. *Journal of Chromatography*, **380**, 407–413.

NASH, T., 1953, The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochemical Journal*, **55**, 416–421.

NEBERT, D. W. and GONZALEZ, F. J., 1987, P450 genes: structure, evolution, and regulation. *Annual Review of Biochemistry*, **45**, 945–993.

NEBERT, D. W. and JONES, J. E., 1989, Regulation of the mammalian cytochrome P_1-450 (CYP1A1) gene. *International Journal of Biochemistry*, **21**, 243–252.

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. and WATERMAN, M. R., 1989, The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromasomal loci. *DNA*, **8**, 1–13.

NEBERT, D. W., PUGA, A. and VASILIOU, V., 1993, Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. *Annals of the New York Acadamy of Science*, **685**, 624–640.

NEDELCHEVA, V. and GUT, I., 1994, P450 in the rat and man: methods of investigation, substrate specificities and relevance to cancer. *Xenobiotica*, **24**, 1151–1175.

NELSON, D. R., KOYMANS, L., KAMATAKI, T., STEGEMAN, J. J., FEYEREISEN, R., WAXMAN, D. J., WATERMAN, M. R., GOTOH, O., COON, M. J., ESTABROOK, R. W., GUNSALAS, I. C. and NEBERT, D. W., 1996, P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, **6**, 1–42.

NELSON, D. R. and STROBEL, H. W., 1987, Evolution of cytochrome P-450 proteins. *Molecular Biology and Evolution*, **4**, 572–593.

OKEY, A. B., 1990, Enzyme induction in the cytochrome P-450 system. *Pharmacology and Therapeutics*, **45**, 241–298.

OMURA, T. and SATO, R., 1964, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *Journal of Biological Chemistry*, **239**, 2370–2378.

PANTUCK, E. J., PANTUCK, C. B., GARLAND, W. A., MIN, B. H., WATTENBERG, L. W., ANDERSON, K. E., KAPPAS, A. and CONNEY, A. H., Stimulatory effect of brussels sprouts and cabbage on human drug metabolism. *Clinical Pharmacology and Therapeutics*, **25**, 88–95.

PARKE, D. V., 1990, Induction of cytochromes P-450—general principles and biological consequences. In *Frontiers of Biotransformation*, vol. 2, edited by K. Ruckpaul and H. Rein (London: Taylor & Francis), pp. 1–34.

PASCO, D. S., BOYUM, K. W., MERCHANT, S. N., CHALBERG, S. C. and FAGAN, J. B., 1988, Transcriptional and post-transcriptional regulation of the genes encoding cytochromes P-450c and P-450d *in vivo* and in primary hepatocyte cultures. *Journal of Biological Chemistry*, **263**, 8671–8676.

PETERSEN, K.-U., 1995, Review article: omeprazole and the cytochrome P450 system. *Alimentary Pharmacology and Therapeutics*, **9**, 1–9.

PUGA, A., RAYCHAUDHURI, B., SALATA, K., ZHANG, Y. H. and NEBERT, D. W., 1990, Stable expression of mouse Cyp1a1 and human CYP1A2 cDNAs transfected into mouse hepatoma cells lacking detectable P450 enzyme activity. *DNA and Cell Biology*, **9**, 425–436.

RAAFLAUB, J. and DUBACH, U. C., 1975, On the pharmacokinetics of phenacetin in man. *European Journal of Clinical Pharmacology*, **8**, 261–265.

RAYCHAUDHURI, B., NEBERT, D. W. and PUGA, A., 1990, The murine *Cyp1a-1* gene negatively regulates its own transcription and that of other members of the aromatic hydrocarbon-responsive [*Ah*] gene battery. *Molecular Endocrinology*, **4**, 1773–1781.

REIK, M., ROBERTSON, R. W., PASCO, D. S. and FAGAN, J. B., 1994, Down-regulation of nuclear aryl hydrocarbon receptor DNA-binding and transactivation functions: requirement for a labile or inducible factor. *Molecular Cell Biology*, **14**, 5653–5660.

REKKER, R. F., TER LAAK, A. M. and MANNHOLD, R., 1993, On the reliability of calculated log*P*-values: Rekker, Hansch/Leo and Suzuki approach. *Quantitative Structure–Activity Relationships*, **12**, 152–157.

RYAN, D. E., THOMAS, P. E. and LEVIN, W., 1980, Hepatic microsomal cytochrome P-450 from rats treated with isosafrole. *Journal of Biological Chemistry*, **255**, 7941–7955.

RYU, D.-Y., LEVI, P. E., and HODGSON, E., 1995, Regulation of cytochrome P-450 isozymes CYP1A1, CYP1A2 and CYP2B10 by three benzodioxole compounds. *Chemico-Biological Interactions*, **96**, 235–247.

SESARDIC, D., BOOBIS, A. R., EDWARDS, R. J. and DAVIES, D. S., 1988, A form of cytochrome P-450 in man, orthologous to form d in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. *British Journal of Clinical Pharmacology*, **26**, 363–372.

SESARDIC, D., BOOBIS, A. R., MURRAY, B. P., MURRAY, S., SEGURA, J., DE LA TORRE, R. and DAVIES, D. S., 1990a, Furafylline is a potent and selective inhibitor of cytochrome P450IA2 in man. *British Journal of Clinical Pharmacology*, **29**, 651–663.

SESARDIC, D., PASANEN, M., PELKONEN, O. and BOOBIS, A. R., 1990b, Differential expression and regulation of the cytochrome P450IA gene subfamily in human tissues. *Carcinogenesis*, **11**, 1183–1188.

SHIMADA, T., YAMAZAKI, H., MIMURA, M., INUI, Y. and GUENGERICH, F. P., 1994, Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *Journal of Pharmacology and Experimental Therapeutics*, **270**, 414–423.

TARRÚS, E., CAMI, J., ROBERTS, D. J., SPICKETT, R. G. W., CELDRAN, E. and SEGURA, J., 1987a, Accumulation of caffeine in healthy volunteers treated with furafylline. *British Journal of Clinical Pharmacology*, **23**, 9–18.

TARRÚS, E., GARCIA, I., ROBERTS, D. J. and SEGURA, J., 1987b, Animal model for the detection of drug-induced inhibition of caffeine metabolism. *Methods and Findings in Experimental Clinical Pharmacology*, **9**, 311–316.

THOMAS, P. E., REIK, L. M., RYAN, D. E. and LEVIN, W., 1983, Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochromes P-450c and P-450d, by structurally diverse xenobiotics. *Journal of Biological Chemistry*, **258**, 4590–4598.