Up-regulation of CYP1A/B in rat lung and liver, and human liver precision-cut slices by a series of polycyclic aromatic hydrocarbons; association with the Ah locus and importance of molecular size

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Abstract

Exposure of precision-cut rat liver slices to six structurally diverse polycyclic aromatic hydrocarbons, namely benzo[a]pyrene, benzo[b]fluoranthene, dibenzo[a,h]anthracene, dibenzo[a,l]pyrene, fluoranthene and 1-methylphenanthrene, led to induction of ethoxyresorufin *O*-deethylase, CYP1A apoprotein and CYP1A1 mRNA levels, but to a markedly different extent. In liver slices, constitutive CYP1A1 mRNA levels were higher, as well as being markedly more inducible by PAHs, compared with CYP1B1, a similar profile to that observed in human liver slices following exposure to the PAHs. Increase in ethoxyresorufin *O*-deethylase and in CYP1A1 apoprotein levels was also observed when precision-cut rat lung slices were incubated with the same PAHs, the order of induction potency being similar to that observed in liver slices. Under the same conditions of exposure, CYP1B1 apoprotein levels were elevated in the lung. Up-regulation of CYP1A1 by the six PAHs correlated with their affinity for the Ah receptor, determined using the chemical-activated luciferase expression (CALUX) assay. It may be concluded that: (a) precision-cut liver and lung slices may be used to assess the CYP1 induction potential of chemicals at the activity, apoprotein and mRNA levels; (b) rat is a promising surrogate animal for human in studies to evaluate CYP1 induction potential; (c) CYP1A1 is far more inducible than CYP1B1 in both rat liver and lung; (d) CYP1 up-regulation by PAHs is related to their affinity for the Ah receptor, and finally (e) computer analysis revealed that the ratio of molecular length/width is an important determinant of CYP1 induction potency among equi-planar PAHs.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise one of the largest and most ubiquitous classes of environmental chemical carcinogens. The major sources of human exposure are diet, as these are formed during domestic cooking, air and tobacco smoking (Skog and Jägerstad, 1998). They are indirect-acting genotoxic carcinogens in that they manifest their carcinogenicity through reactive intermediates that are produced following metabolic activation catalysed in many tissues, but most prominently in the liver.

The principal pathway of metabolic activation of PAHs proceeds through an initial cytochrome P450-mediated bioactivation to generate epoxides which are converted to the corresponding *trans*-dihydrodiols by epoxide hydrolase; finally, a second oxidation, also catalysed by cytochromes P450, yields the dihydrodiol-epoxide, the ultimate carcinogen (Conney, 1982). The cytochrome P450 family responsible for the metabolism, including bioactivation, of PAHs is CYP1, in particular CYP1A1 and CYP1B1 (Shimada and Fujii-Kuriyama, 1995; Ioannides and Lewis, 2004). Both of these enzymes are constitutively poorly expressed in the liver, and are essentially extrahepatic enzymes (Guengerich, 1990; Bhattacharyya et al., 1995). CYP1, however, is probably the most inducible CYP family, at least in terms of induction observed, being up-regulated by planar compounds in the liver and extrahepatic tissues of animals and humans (Ioannides and Parke, 1990; Christou et al., 1995). This up-regulation of CYP1 is regulated by the Ah (aryl hydrocarbon) receptor, and a number of studies have shown that CYP1-inducing PAHs are good ligands for this receptor (Cheung et al., 1993; Machala et al., 2001; Piskorska-Plizczynska et al., 1986).

The current studies were conducted in precision-cut slices in order to facilitate the use of human tissue. The advantages of adopting this *in vitro* system, in comparison with other systems such as subcellular fractions and primary hepatocytes, have frequently been pointed out in reviews (de Kanter et al., 1999; Lerche-Langrand and Toutain, 2000). Slices have been prepared from a number of tissues including liver (Hashemi et al., 1999), lung (Umachandran et al., 2004), intestine and colon (van de Kerkhof et al., 2005).

The objectives of the present study were to: (a) assess the ability of six structurally diverse PAHs to up-regulate the two CYP1 enzymes involved in their metabolism, namely CYP1A1/B1, in the liver and lung, the principal site of their bioactivation and a target tissue respectively; (b) establish whether precision-cut liver slices can be used to assess the potential of PAHs to modulate CYP1A1/B1; (c) evaluate whether rat is an appropriate surrogate animal for human in such studies by investigating the ability of the same series of PAHs to modulate CYP1A1 in human liver slices; (d) investigate whether up-regulation of the same enzyme systems involves transcriptional activation mediated through the Ah receptor, and finally (e) through computer analysis identify structural characteristics that are associated with the induction of the CYP1 enzymes.

Materials and methods

Materials

Dibenzo[a,l]pyrene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1-methylphenanthrene (LGC Promochem, Middlesex, UK), rat genomic DNA (Novagen, Wisconsin, USA), RNase-free DNase, cell culture lysis reagent, luciferase assay reagent (Promega, Wisconsin, USA), NADPH, sulphatase, β -glucuronidase, β -

naphthoflavone, benzo[a]pyrene, fluoranthene, benzo[b]fluoranthene, dibenzo[a,h]anthracene, ethoxyresorufin, resorufin, 7-ethoxycoumarin, peroxidase-linked anti-rabbit, anti-goat and anti-sheep antibodies (Sigma Co. Ltd., Poole, Dorset, UK), Qiagen RNeasy Mini kits (Crawley, West Sussex, UK), AbsoluteTM QPCR Mix (Abgene, Epsom, Surrey, UK), and Earle's balanced salt solution (EBSS), foetal calf serum, gentamycin, hexamers, Superscript II and RPMI 1640 with L-glutamine culture medium (Invitrogen, Paisley, Scotland) were all purchased. Twelve-well plates were obtained from Bibby Sterilin (Helena Biosciences, Sunderland, UK). Rat anti-CYP1A1, recognising both CYP1A1 and CYP1A2, and anti-CYP1B1 antibodies were obtained from BD Biochemicals (Oxford, UK).

Preparation and incubation of precision-cut tissue slices

Liver sections from two human cadaveric livers that could not be used for transplantation purposes were obtained from the UK Human Tissue Bank (The Innovation Centre, Oxford Street, Leicester, U.K). Donor 1 was a 60-year old male and Donor 2 a 57-year old female; both were Caucasian and smokers. Sections were received 8 - 12 hours after the liver was removed from the donor and were transported in cold University of Wisconsin (UW) preservation solution on ice. On receipt, the liver sections were immediately transferred into a sterile container and, after the UW solution was carefully decanted, they were washed 3 to 4 times with culture medium. Slices were prepared as with rat liver (*vide infra*) and pre-incubated for 30 minutes prior to the start of the experiment, to allow equilibrium to be reached and ensure complete removal of the transport buffer. Metabolic viability was evaluated using 7-ethoxycoumarin as substrate, following a 6-hour incubation (*vide infra*). Male Wistar albino rats (200g) were obtained from B&K Universal Ltd (Hull, East Yorkshire,

UK). The animals were housed at 22 ± 2 °C, 30-40 % relative humidity in an alternating 12-hr light:dark cycle with light onset at 07.00 hr. Rats were killed by cervical dislocation, and liver was immediately excised. In the *in vivo* study, rats were treated with a single intraperitoneal dose of either β -naphthoflavone or benzo[a]pyrene (25 mg/kg) and were killed 24 hours afterwards.

Rat and human liver slices (250 μ m) were prepared from 8mm cylindrical cores using a Krumdieck tissue slicer (Alabama Research and Development Corporation, Munsford, AL, USA) as previously described (Hashemi et al., 1999). The multiwell plate procedure, using 12-well culture plates, was used to culture the slices. One slice was placed in each well, in 1.5 ml of culture medium. Slices were incubated under sterile conditions on a reciprocating plate shaker housed in a humidified incubator, at a temperature of 37 °C and under an atmosphere of 95 % air/5% CO₂. The slices were initially pre-incubated for 30 min in order to slough off any dead cells due to slicing. For the production of lung slices, animals were killed by an overdose of sodium pentobarbital, and lungs were perfused intratracheally with agarose (0.75% v/w) at 37 °C. Agar was allowed to solidify, and lung slices (600 μ m) were prepared from cylindrical cores (8 mm) as described for the liver (Umachandran et al., 2004, 2006). A pre-incubation of 60 minutes was carried out. For incubations exceeding 24 h, slices were placed in fresh medium every 24 h. Three different slice pools, comprising 4-10 slices, were used per time point.

Enzyme assays

Following incubation, slices were removed from the medium, homogenised, and microsomal fractions were prepared by differential centrifugation. The *O*-deethylation of ethoxyresorufin (Burke and Mayer, 1974) was determined in hepatic microsomes,

but in the lung studies activity was determined in the post-mitochondrial fraction (S9), as it was more facile to determine the low levels of activity in lung slices following a 24-hour incubation; in this instance the incubation system was supplemented with dicoumarol (8 μ M) in order to inhibit cytosolic quinone reductase (Price et al., 2004). Protein concentration was determined in both fractions (Bradford, 1976). Finally, in order to determine apoprotein levels, hepatic microsomal proteins were resolved by electrophoresis and incubated with the primary antibody and the corresponding peroxidase-linked secondary antibody. Immunoblots were quantitated by densitometry using the GeneTool software (Syngene Corporation, Cambridge, UK). In preliminary studies, a linear response was established with protein levels ranging 50 to 400 μ g of protein (results not shown).

Transcript level measurement

Two slices were used for total RNA extraction, and for each sample triplicates were carried out. RNA was extracted using the Qiagen RNeasy Mini kit and was quantified using a Nanodrop spectrophotometer. Total RNA was treated with RNase-free DNase to remove genomic contamination. Reverse transcription was primed with random hexamers and carried out by Superscript II according to the manufacturer's instructions. To ensure that DNase-treated samples were free from genomic contamination, an RT- control was carried out for every RNA sample. cDNA generated from 50ng was amplified using AbsoluteTM QPCR Mix with 400 nM primers and 100 nM fluorogenic probe in a total reaction volume of 25 μ l. Q-PCR reactions were run on the ABI7000 SDS instrument (Applied Biosystems, Warrington, UK) and quantitation was carried out using the ABI proprietary software against a standard curve generated from rat genomic DNA.

For the quantitative reverse transcription-polymerase chain reaction, the primers and TAMRA/FAM dual labelled probes (Table 1) were designed using the Primer Express software (Applied Biosystems) and purchased from MWG, Ebersberg, Germany. Each primer and probe set was designed to amplify sequences within a single exon, so that genomic DNA could be used as a standard.

Metabolism of 7-ethoxycoumarin by precision-cut human liver slices

The metabolism of 7-ethoxycoumarin (50 μ M) by human liver slices to 7-hydroxycoumarin and to its sulphate and glucuronide conjugates was monitored as described by Steensma et al. (1994). For the determination of the conjugates, aliquots of the media were diluted with a half-volume of 0.5 M sodium acetate buffer (pH 5), containing either β -glucuronidase (5000 Units/ml) or sulphatase (250 Units/ml) containing the β -glucuronidase inhibitor D-saccharic acid 1,4-lactone (17mM). The mixtures were incubated for 16 h at 37 °C, and the free 7-hydroxycoumarin was determined as follows. Aliquots (0.3 ml) of the incubation media, before and after deconjugation, were made to 1 ml with 0.154 M KCl buffer containing 50 mM Tris-HCl, pH 7.4. Following addition of 4M HCl (0.25 ml), the samples were extracted for 30 min with chloroform (6 ml) on a rotary mixer. Aliquots (5 ml) of the chloroform layer were subsequently extracted for a further 30 min with 3ml of 0.5 M glycine-NaOH buffer, pH 10.5. The fluorescence of the aqueous layer was measured using excitation and emission wavelengths of 380 and 452 nm respectively. Standards of 7-hydroxycoumarin were run through the same procedure. Finally, slices were homogenised in 0.154 M KCl buffer containing 50 mM Tris-HCl, pH 7.4, and total protein was determined using bovine serum albumin as standard (Bradford, 1976).

AhR ligand-binding assay

Interactions of the PAHs with the Ah receptor were assessed using the chemical-activated luciferase expression (CALUX) assay. In 24-well plates, H1L1.1c2 cells were cultured (7×10^4 cells/ml) in α -MEM (minimum essential medium) supplemented with 10% FBS and penicillin-streptomycin-neomycin antibiotic solution; cells were cultured for 24 hours until 50-70 % confluent. Cells were then incubated with a range of concentrations of PAHs, dissolved in DMSO, for 24 hours at 37°C and 5 % CO₂ in a humid environment, and subsequently washed with PBS; an aliquot of the cell culture (100 μ l) was incubated for 15 minutes with the lysis reagent. Cell lysates were centrifuged at 13000g for 2 minutes, and luciferase activity in the supernatant was determined using the Promega stabilised luciferase assay reagent according to the manufacturer's instructions. Luminescence was read in a Packard Lumicount microplate luminometer with PlateReader software (Packard Instrument Company). TCDD (10^{-9} M) served as a positive control, achieving 100 % binding.

In silico molecular analysis

Electronic structural parameters were determined using the AM1 molecular orbital method based on previously minimised molecular structures. Molecular shape parameters (area/depth² and length/width) were produced from the dimensions (length, width and depth) of each molecule as described previously (Lewis, et al., 1998; 2002). All structural calculations were conducted on the Sybyl 7.0 (Tripos Associates, St. Louis, MO) molecular modelling suite of programs operating under Linux.

Statistical evaluation

Statistical evaluation was carried out using the Student's t-test.

Results

The structures of the PAHs employed in the current study are shown in Figure 1.

Rat liver studies

Following exposure of rat liver slices to a range of concentrations (1-100 μ M) of PAHs for 24 hours, the *O*-deethylation of ethoxyresorufin was markedly induced by benzo[a]pyrene and dibenzo[a,h]anthracene, maxima being achieved at about 10 μ M; extent of induction declined at higher concentrations (Figure 2). In the case of benzo[b]fluoranthene it was observed that maximum induction had already been achieved at the lowest concentration used i.e. 1 μ M (results not shown) so an additional study was undertaken utilising a lower range of concentrations (0.25-1 μ M); a concentration-dependent increase in ethoxyresorufin *O*-deethylase was observed (Figure 2). Finally, 1-methylphenanthrene and fluoranthene failed to modulate this enzyme activity whereas dibenzo[a,l]pyrene caused significant, but modest, increase only at the 10 μ M concentration (Figure 2).

Immunoblot analysis following probing of microsomes with rat anti-CYP1A1, showed that the changes in ethoxyresorufin *O*-deethylase following exposure to benzo[a]pyrene, benzo[b]fluoranthene and dibenzo[a,h]anthracene were paralleled by similar changes in apoprotein levels, and a less pronounced rise in apoprotein levels was seen with dibenzo[a,l]pyrene (Figure 3). Fluoranthene also showed a rise in

apoprotein levels, albeit at the higher concentrations, but 1-methylphenanthrene had no significant effect (Figure 3).

All PAHs studied led to an increase in CYP1A1 mRNA levels when incubated with precision-cut liver slices for 24 hours (Figure 4). A marked rise in mRNA levels was evident with benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[b]fluoranthene and, to a lesser extent, dibenzo[a,l]pyrene at the lowest concentration studied, i.e. 0.1 μ M, but in the case of fluoranthene and 1-methylphenanthrene concentrations of 5 and 10 μ M respectively were required for a statistically significant increase to be manifested (Figure 4).

As there is no selective substrate suitable for monitoring CYP1B1 activity (Murray *et al.*, 2001), modulation of this activity in the lung was assessed at the apoprotein level. However, as a result of the poor expression of CYP1B1 in the liver (Bhattacharyya *et al.*, 1995), apoprotein levels were difficult to detect, especially after incubation of slices for 24 hours during which cytochromes P450 decline (Hashemi *et al.*, 2000), and was monitored at the mRNA level. When CYP1B1 mRNA levels were monitored, once again a rise was noted in liver slices exposed to all PAHs, with benzo[b]fluoranthene, benzo[a]pyrene and dibenzo[a,h]anthracene being the more potent (Figure 5). Constitutive levels of CYP1B1 mRNA levels were much lower compared with CYP1A1 (51 ± 5 copies per 50 ng RNA compared to 8623 ± 231 in the case of CYP1A1 mRNA).

Rat lung studies

Exposure of precision-cut rat lung slices to the various PAHs for 48 hours led to enhancement of ethoxyresorufin *O*-deethylase activity but, as observed in liver slices, there was marked difference in potency; benzo[a]pyrene, benzo[b]fluoranthene and

dibenzo[a,h]anthracene were the most effective, whereas dibenzo[a,l]pyrene was a modest inducer, and fluoranthene and 1-methylphenanthrene failed to significantly modulate this activity (Figure 6). In all cases induction maxima were reached at the 1 μ M concentration.

Immunoblot analysis employing antibodies to CYP1A1 revealed marked changes in the CYP1A1 apoprotein levels that paralleled activity, except in the case of 1-methylphenanthrene where a relatively modest increase in apoprotein levels in the absence of a significant rise in ethoxyresorufin *O*-deethylase activity was observed (Figure 7).

When the immunoblot studies were conducted utilising antibodies to CYP1B1, only modest increases, compared with CYP1A1, in apoprotein levels were revealed when rat lung slices were incubated with the PAHs under identical conditions (Figure 8). Benzo[a]pyrene, benzo[b]fluoranthene and dibenzo[a,h]anthracene were the most potent inducers, whereas fluoranthene and dibenzo[a,l]pyrene did not display a major effect. 1-Methylphenanthrene, however, elevated CYP1B1 apoprotein levels when incubated with the lung slices at high concentrations, as observed with CYP1A1.

Human liver studies

The described studies were conducted on two different occasions, i.e. using two different fresh liver samples. Liver metabolic viability of human slices was monitored using 7-ethoxycoumarin as the model substrate. Although both liver samples converted 7-ethoxycoumarin to the sulphate and glucuronide conjugates of 7-hydroxycoumarin, the rate of metabolism of the liver from donor 1 was double that of donor 2 (Figure 9); donor 1 also displayed higher ethoxyresorufin *O*-deethylase activity. However, when slices from both livers were exposed to benzo[a]pyrene,

induction potency per μmole of ethoxyresorufin *O*-deethylase was similar, being 6.58 and 6.02 for donors 1 and 2 respectively (results not shown). Exposure of liver slices to PAHs modulated ethoxyresorufin *O*-deethylase activity; the most potent inducers were benzo[a]pyrene, dibenzo[a,h]anthracene whereas dibenzo[a,l]pyrene and fluoranthene displayed a relatively modest effect, and 1-methylphenanthrene had no significant effect (Figure 10). In the case of benzo[b]fluoranthene maximum elevation in enzyme activity appears to have been attained at a concentration of 1 μM , the lowest concentration studied. Immunoblot analysis revealed similar changes in apoprotein levels (Figure 11).

Ex-vivo study

Antibodies to CYP1B1 immunoreacted with a single band in both rat liver and lung microsomes. Treatment with benzo[a]pyrene elevated apoprotein levels only in the lung whereas β -naphthoflavone had no effect in either tissue at this dose level (Figure 12)

Binding to the Ah receptor

The six PAHs differed markedly in their ability to bind to the Ah receptor, with EC_{50} values covering at least four orders of magnitude. Benzo[a]pyrene, dibenzo[a,h]anthracene and, especially, benzo[b]fluoranthene were the best ligands (Figure 13, Table 2), whereas 1-methylphenanthrene exhibited no significant binding.

Computer analysis

Table 3 lists the molecular and electronic parameters of the six PAHs used in this study, which have been employed to identify correlations with CYP1 induction

potency. With the exception of 1-methylphenanthrene, all PAHs were planar compounds having the same depth (D) and characterised by a large a/d^2 value.

Discussion

The conditions of exposure of slices to PAHs were based on our previous studies (Pushparajah et al., 2007). When CYP1 was monitored at the activity level, using ethoxyresorufin as probe substrate, a marked difference in the ability of the six PAHs to elevate this activity in rat liver slices was noted. The present findings mirror observations in Ah-responsive mice following treatment with a series of PAHs where benzo[b]fluoranthene and dibenzo[a,h]anthracene were among the most potent inducers of ethoxyresorufin *O*-deethylase, benzo[a]pyrene was also a good inducing agent, more effective than dibenzo[a,l]pyrene, whereas fluoranthene was poor (Shimada et al., 2003). Moreover, the present observations make a case for using precision-cut liver slices for assessing induction potential of slices, rather than *in vivo* studies, which not only allow the facile use of many concentrations but also minimise the number of animals. The rise in activity was accompanied by elevation in apoprotein levels indicating that increased enzyme availability is, at least in part, responsible for the enhanced activity. Increased transcription, as evidenced by a rise in CYP1A1 mRNA levels, is likely to contribute to the enhanced enzyme synthesis. All PAHs studied elevated mRNA levels albeit to a different extent, but an increase in protein levels and ethoxyresorufin *O*-deethylase activity were observed with the most potent inducers. It has been suggested that a threshold exists that has to be exceeded in order for mRNA to be translated, so that activation of the gene at the mRNA level does not always lead to higher protein levels, and concurs with the data presented herein (Greenbaum et al., 2003). In recent studies, in agreement with the present

findings, where HepG2 cells and liver slices were exposed to the same series of PAHs and changes in gene expression were assessed using cDNA microarrays and oligonucleotide microarrays respectively, an increase in *CYP1A1* gene expression was noted for all compounds except 1-methylphenanthrene; the most potent were benzo[a]pyrene, benzo[b]fluoranthene and dibenzo[a,h]anthracene (Staal et al., 2006, 2007).

A picture shared by all PAH inducers of ethoxyresorufin *O*-deethylase is that activity decreased at concentrations exceeding those at which maximum induction occurred, although it was still higher than control, and a similar picture emerged when CYP1A apoprotein or mRNA levels were determined. Similar observations have been reported by other workers where exposure of rat liver slices to benzo[a]pyrene caused a more pronounced degree of induction of ethoxyresorufin *O*-deethylase at the lower concentrations studied (Price et al., 2004). A likely explanation is that at these high concentrations viability of slices is compromised leading to impairment of protein synthesis. It may be that the generation of toxic intermediates overwhelms detoxication enzyme systems, such as glutathione *S*-transferase, leading to compromised cellular defences and a rise in toxicity (Whitlock, 1999); it is pertinent to point out that the ethoxyresorufin *O*-deethylase-inducing PAHs, under identical conditions of incubation, cause only a very modest increase in glutathione *S*-transferase activity which, thus, may not be able to compensate for the enhanced generation of oxides and quinones (unpublished observations). However, loss in slice viability was observed by any of the PAHs, at the concentrations employed in the present study, as exemplified by the leakage of lactate dehydrogenase into the medium (results not shown). A likely contributory mechanism may involve inhibition

of CYP1A1 activity at the high concentrations of PAHs (Shimada and Guengerich, 2006).

When human liver slices were likewise exposed to these PAHs, the observed induction profile was very similar to that noted in rat slices. Fluoranthene, however, caused a relatively weak increase in ethoxyresorufin *O*-deethylase activity in human slices, whereas in rat slices it was visible only at the apoprotein and mRNA levels. Immunoblot analysis revealed commensurate increases in apoprotein levels when microsomes were probed with antibodies to CYP1A1, indicating that once again a rise in enzyme levels is responsible for the increased ethoxyresorufin *O*-deethylase activity. However, in contrast to the rat, apoprotein levels did not drop at high PAH concentrations, implying that the decrease in activity observed at high PAH concentrations is due to inhibition rather than impaired enzyme synthesis. It is of interest that fluoranthene and dibenzo[a,l]pyrene, which do not or poorly suppress ethoxyresorufin *O*-deethylase respectively at high concentrations, are poor inhibitors of CYP1A1, whereas benzo[a]pyrene and benzo[b]fluoranthene, which suppress this enzyme activity, are strong inhibitors (Shimada and Guengerich, 2006). The close relationship in ethoxyresorufin *O*-deethylase induction following exposure of human and rat slices to PAHs implies that the human liver Ah receptor is similar to the rat counterpart, at least as far as ligand interaction and subsequent response are concerned, and supports the proposal that the Ah receptor is a highly conserved protein (Bank et al., 1992). Moreover, when hepatocytes were exposed to a series of PAHs, the order of CYP1A induction was the same, but degree of induction was double in rat compared with human (Vakharia et al., 2001; Till et al., 1999). It thus appears that although the affinity for the receptor and, consequently, CYP1A1 inducibility can vary between rat and human, the order of potency for a series of

PAHs is the same, so that studies undertaken in rats to compare the CYP1A induction potential of a series of PAHs can be extrapolated to humans.

Rat lung slices were exposed to the same PAHs, but for 48 hours and at a lower range of concentrations, based on our previous studies (Pushparajah et al., 2007). As we have already reported, substantial variation in the *O*-deethylation of ethoxyresorufin between lung slices was noted, which most likely reflects the fact that the lung, in contrast to the liver, has a heterogeneous cell population, with xenobiotic-metabolising activity being present only in some cell types (Hukkanen et al., 2002). However, it was up-regulated following exposure of the lung slices to the same series of PAHs, the order of induction being similar to that in liver slices. Clearly, what these studies indicate is that no tissue difference exists in CYP1A1 up-regulation by PAHs in rat. In general, extent of induction was more pronounced in the liver; however, maxima in the lung slices were achieved at 1 μ M, whereas in the liver, in most cases, it necessitated a 10 μ M concentration. This difference in concentration may reflect, at least partly, the higher rate of metabolic breakdown of PAHs such as benzo[a]pyrene in liver compared with lung, leading to a rapid decline in its effective concentration; the rate of metabolism of benzo[a]pyrene to phenolic products in liver slices is higher compared with lung slices (unpublished observations). Up-regulation of ethoxyresorufin *O*-deethylase activity in lung slices was accompanied by increase in CYP1A1 apoprotein levels indicating that also in this tissue rise in activity was most likely due to elevated enzyme synthesis. Induction of ethoxyresorufin *O*-deethylase activity declined in lung slices as witnessed in the liver. Concordant to these observations, it has been recently reported that CYP1A1 mRNA levels in lung slices treated with benzo[a]pyrene rose in a concentration-dependent manner up to 10 μ M, but dropped at higher concentrations (Harrigan et al., 2006). A good correlation

has been obtained in the induction of ethoxyresorufin *O*-deethylase activity in the liver and lung, showing that up-regulation of CYP1A is similar in these tissues (Figure 14). When CYP1A levels were monitored immunologically, a far more potent effect was witnessed in the lung. This can be attributed to the fact that the antibody recognises both CYP1A1 and A2, but the latter is only expressed in the liver so that control levels are much higher.

Although the up-regulation of CYP1A1 by PAHs has been extensively studied (Ioannides and Parke, 1990), CYP1B1 received much less attention since it was discovered decades after CYP1A1 (Murray et al., 2001). It is a very important catalyst of the bioactivation of PAHs; of the three members of the CYP1 family, CYP1B1 was the most efficient in converting benzo[a]pyrene to its 7,8-diol, benzo[c]phenanthrene to its 3,4-dihydrodiol, the precursors of the ultimate carcinogens, dibenz[a]anthracene to the 3,4-oxide, the proximate carcinogen, and dibenzo[a,l]pyrene to DNA-binding adducts (Shimada et al., 1996; Luch et al., 1998). All PAHs elevated CYP1B1 mRNA levels in the liver, but the extent of induction was drastically lower compared with CYP1A1 mRNA, indicating that the latter enzyme is far more inducible by this class of compounds in rat liver. Similar observations were made in rats following treatment with TCDD (Walker *et al.*, 1998) and, moreover, exposure of rat liver and lung slices to benzo[a]pyrene led to a more marked increase in CYP1A1 mRNA levels compared with CYP1B1 mRNA (Harrigan et al., 2004). Once again, dibenzo[a,h]anthracene, benzo[b]fluoranthene and benzo[a]pyrene were the most potent, with dibenzo[a,l]pyrene being the least potent; unexpectedly, however, 1-methylphenanthrene was an effective inducer of CYP1B1 mRNA levels. In the lung, with the exception of fluoranthene and dibenzo[a,l]pyrene, all other PAHs studied elevated CYP1B1 apoprotein levels. Once again, 1-methylphenanthrene caused a

marked increase in CYP1B1 apoprotein levels. This PAH is not a ligand for the Ah receptor and raises the possibility that an Ah-independent pathway for the regulation of CYP1B1 may exist (Murray et al., 2001). Indeed, it has already been reported that in Ah receptor knockout mice piperonyl butoxide raised CYP1B1, but not CYP1A1, mRNA levels (Ryu et al., 1996). Since changes in CYP1 expression following exposure to PAHs were monitored at the mRNA and protein level in the liver and lung respectively, thus not allowing direct comparison, a limited *ex vivo* study was conducted where animals were treated with benzo[a]pyrene, the prototype PAH, as well as β -naphthoflavone, an established CYP1 inducer. In both tissues a single immunoreacting band was detected but expression in the lung was more than double that of the liver. Moreover, exposure to β -naphthoflavone failed to up-regulate CYP1B1 apoprotein levels in both tissues whereas benzo[a]pyrene stimulated apoprotein levels in the lung but not liver; it may be cautiously inferred that PAHs as a class may be more potent inducers of CYP1B1 in the lung compared with the liver.

Induction of CYP1 expression by the PAHs, in both human and rat tissues, paralleled the affinity of the PAH for the Ah receptor as previously shown using a series of chrysenes (Cheung et al., 1993) and, moreover, the rise in mRNA levels implies enhanced transcriptional activity. *In vitro* studies employing tissue slices offer an advantage over *in vivo* studies in establishing such correlations in that in the former approach tissue concentration is not influenced by pharmacokinetic processes such as absorption and pre-systemic metabolism. Inducers of the CYP1A subfamily are essentially planar molecules characterised by a low depth and high area/depth² ratio, an index of planarity (Lewis et al., 1986). Of the six PAHs studied, only 1-methylphenanthrene is not completely planar because of the methyl substitution and this may explain its inability to ligand to the Ah receptor. However, even non-

substituted tricyclic PAHs such as anthracene, pyrene and fluorene (Ayrton et al., 1990; Shimada et al., 2002; Piskorska-Plizczynska et al., 1986; Machala et al., 2001) are poor ligands of the Ah receptor and at best weak CYP1A inducers, raising the possibility that molecular size is an important factor. For this reason, molecular size and electronic parameters were calculated for each PAH used in the current studies. No relationship could be found between any single electronic parameter calculated and CYP1A induction activity as exemplified by the *O*-deethylation of ethoxyresorufin. However, a good correlation has been obtained between CYP1A1 inducibility in both liver and lung and the ratio of length/width even using this small number of compounds (Figure 15); PAHs with a small ratio were poor CYP1 inducers and ligands to the Ah receptor. Similar good correlation has been obtained between the same ratio and increase in hepatic CYP1A1 and CYP1B1 mRNA levels (Figure 15). These observations are in agreement with our previous studies where benzo[a]pyrene and 2-acetylaminofluorene were good inducers of CYP1, whereas their isomers benzo[e]pyrene and 4-acetylaminofluorene, having a lower length/width ratio, were poor inducers of this enzyme (Lewis et al., 1994). It is relevant to point out that TCDD, the most avid ligand identified for the Ah receptor, has a length/width ratio of 1.889 and possibly reflects the optimum dimensions.

In conclusion, the current studies have confirmed that CYP1A induction can be monitored in lung and liver slices and, moreover, demonstrated that: (a) precision-cut liver and lung slices can be used also to assess the inducibility of CYP1B1; (b) in rat liver and lung PAHs induce both CYP1A/CYP1B1, but to a markedly different extent; (c) at the mRNA level, hepatic CYP1A is far more inducible than CYP1B1 by PAHs; (d) CYP1 induction by PAHs is correlated to their ability to interact with the Ah receptor; (e) CYP1A1 activity is induced by PAHs to a similar extent in rat and

human liver slices indicating that the rat is an appropriate surrogate animal for human in such studies, and (f) in equiplanar PAHs, molecular dimensions are important factors in determining CYP1 up-regulation.

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References

- Ayrton, A.D., 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.
- Bank, P.A., Yao, E.F., Phelps, C.L., Harper, P.A. and Denison, M.S. (1992) Species-specific binding of transformed Ah receptor to a dioxin responsive transcriptional enhancer. *Eur. J. Pharmacol.* 228, 85-94.
- Bhattacharyya, K.K., Brake, P.B., Eltom, S.E., Otto, S.A. and Jefcoate, C.R. (1995) Identification of a rat adrenal cytochrome P450 active in polycyclic hydrocarbon metabolism as rat CYP1B1. Demonstration of a unique tissue-specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. *J Biol Chem.* **270**, 11595-11602.
- Bradford, M.M. (1976) A rapid and sensitive method for the quantitation on microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.
- Burke, M.D. and Mayer, R.T. (1974) Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab. Disp.* 2, 583-588.

Cheung, Y.L., Gray, T.J. and Ioannides, C. (1993) Mutagenicity of chrysene, its methyl and benzo derivatives, and their interactions with cytochromes P-450 and the Ah-receptor; relevance to their carcinogenic potency. *Toxicology* 81, 69-86.

Christou, M., Savas, U., Schroeder, S., Shen, X., Thompson, T., Gould, M.N. and Jefcoate, C.R. (1995) Cytochromes CYP1A1 and CYP1B1 in the rat mammary gland: Cell-specific expression and regulation by polycyclic aromatic hydrocarbons and hormones, *Mol. Cell. Endocrinol.* 115, 41-50.

Conney, A.H. (1982) Induction of microsomal-enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic-hydrocarbons - Clowes, G.H.A. memorial lecture. *Cancer Res.* 42, 4875-4917.

De Kanter, R., Olinga, P., De Jager, M.H., Merema, M.T., Meijer, D.K.F. and Groothuis, G.M.M. (1999) Organ slices as an *in vitro* test system for drug metabolism in human liver, lung and kidney. *Toxicol. In Vitro* 13, 737-744.

Greenbaum, D., Colangelo, C., Williams, K., and Gerstein, M. (2003) Comparing protein abundance and mRNA expression levels on a genomic scale. *Genom. Biol.* 4, 117-23.

Guengerich, F.P. (1990) Characterisation of roles of human CYP450 enzymes in carcinogen metabolism. *Asia Pacific J. Pharmacol.* 5, 327-45.

Harrigan, H.A., McGarrigle, B.P., Sutter, T.R. and Olson, J.R. (2004) Tissue specific induction of cytochrome P450 (CYP) 1A1 and 1B1 in rat liver and lung following in vitro (tissue slice) and in vivo exposure to benzo[a]pyrene. *Toxicol. In vitro* 20, 426-438.

Hashemi, E., Dobrota, M., Till, C. and Ioannides, C. (1999) Structural and functional integrity of precision-cut liver slices in xenobiotic metabolism: a comparison of the dynamic organ and multiwell plate culture procedures. *Xenobiotica* 29, 11-25.

Hashemi, E., Till, C. and Ioannides, C. (2000) Stability of cytochrome P450 proteins in cultured precision-cut rat liver slices. *Toxicology* 149, 51-61.

Hukkanen, J., Pelkonen, O., Hakkola, J. and Raunio, H. (2002) Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit. Rev. Toxicol.* 32, 391-411.

Ioannides, C. and Lewis, D.F.V. (2004) Cytochromes P450 in the bioactivation of chemicals. *Curr. Topics Medicin. Chem.* 4, 1767-1788.

Ioannides, C. and Parke, D.V. (1990) The cytochrome P450 I gene family of microsomal haemoproteins and their role in the metabolic activation of chemicals. *Drug Met. Rev.* 22, 1-85.

Lerche-Langrand, C. and Toutain, H.J. (2000) Precision-cut liver slices: characteristics and use for in vitro pharmaco-toxicology. *Toxicology* 153, 221-253.

Lewis, D.F.V., Ioannides, C. and Parke, D.V. (1986) Molecular dimensions of the substrate binding site of cytochrome P-448. *Biochem. Pharmacol.* 35, 2179-2185.

Lewis, D.F., Ioannides, C. and Parke, D.V. (1994) Molecular modelling of cytochrome CYP1A1: a putative access channel explains differences in induction potency between the isomers benzo(a)pyrene and benzo(e)pyrene, and 2- and 4-acetylaminofluorene. *Toxicol. Lett.* 71, 235-43.

Lewis, D.F.V., Ioannides, C. and Parke, D.V. (1998) An improved and updated version of the COMPACT procedure for the evaluation of P450-mediated chemical activation. *Drug Met. Rev.* 30, 709-737.

Lewis, D.F.V., Bird, M.G. and Jacobs, M.N. (2002) Human carcinogens: an evaluation study via the COMPACT and HazardExpert procedures. *Human Exp. Toxicol.* 21, 115-122.

Luch, A., Coffing, S.L., Tang, Y.M., Schneider, A., Soballa, V., Greim, H., Jefcoate, C.R., Seidel, A., Greenlee, W.F., Baird, W.M. and Doehmer, J. (1998) Stable expression of human cytochrome P450 1B1 in V79 Chinese hamster cells and metabolically catalyzed DNA adduct formation of dibenzo[a,l]pyrene. *Chem. Res. Toxicol.* 11, 686-695.

Machala, M., Vondracek, J., Blaha, L., Ciganek, M. and Neca, J. (2001) Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using *in vitro* reporter gene assay. *Mutat. Res.* 497, 49-62.

Murray, G.I., Melvin, W.T., Greenlee, W.F., and Burke, M.D. (2001) Regulation, function, and tissue-specific expression of cytochrome P450 CYP1B1. *Annu. Rev. Pharmacol. Toxicol.* 41, 297-316.

Piskorska-Pliszczyńska, J., Keys, B., Safe, S. and Newman, M.S. (1986) The cytosolic receptor binding affinities and AHH induction potencies of 29 polynuclear aromatic hydrocarbons. *Toxicol Lett.* 34, 67-74.

Price, R.J., Renwick, A.B., Walters, D.G., Young, P.J. and Lake, B.G. (2004) Metabolism of nicotine and induction of CYP1A forms in precision-cut rat liver and lung slices. *Toxicol In Vitro* 18, 179-85.

Pushparajah, D., Umachandran, M., Plant, K.E., Plant, N. and Ioannides, C. (2007) Evaluation of the precision-cut liver and lung slice systems for the study of induction of CYP1, epoxide hydrolase and glutathione *S*-transferase activities. *Toxicology* 231, 68-80.

Ryu, D.Y., Levi, P.E., Fernandez Salguero, P., Gonzalez, F.J. and Hodgson, E. (1996) Piperonyl butoxide and acenaphthylene induce cytochrome P450 1A2 and 1B1 mRNA in aromatic hydrocarbon-responsive receptor knock-out mouse liver. *Molec. Pharmacol.* 50, 443-446.

Shimada, T. and Fujii-Kuriyama, Y. (2004) Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer Sci.* 95,1-6.

Shimada, T. and Guengerich, P.F. (2006) Inhibition of human cytochrome P450 1A1-, 1A2-, and 1B1-mediated activation of procarcinogens to genotoxic metabolites by polycyclic aromatic hydrocarbons. *Chem. Res. Toxicol.* 19, 288-294.

Shimada, T., Hayes, C.L., Yamazaki, H., Amin, S., Hecht, S.S., Guengerich, P.F. and Sutter, T.R. (1996) Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* 56, 2979-2984.

Shimada, T., Inoue/ K., Suzuki, Y., Kawai, T, Azuma, E., Nakajima, T., Shindo. M., Kurose, K, Sugie, A., Yamagishi, Y., Fujii-Kuriyama, Y. and Hashimoto, M. (2002) Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1,1A2 and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/ 6J mice. *Carcinogenesis* 23, 1199-1207.

Shimada, T., Sugie, A., Yamada, T., Kawazoe, H., Hashimoto, M., Azuma, E., Nakajima, T., Inoue, K. and Oda. Y. (2003) Dose-response studies on the induction of liver cytochromes P4501A1 and 1B1 by polycyclic aromatic hydrocarbons in arylhydrocarbon-responsive C57BL/6J mice. *Xenobiotica* 33, 957-71.

Skog, K.I. and Jägerstad, M. (1998) Toxicants in food: Generated during cooking In: C. Ioannides. (Ed.), Nutrition and Chemical Toxicity, John Wiley & Sons, Chichester, UK, pp. 59-79.

Staal, Y.C.M., van Herwijnen, H.M., van Schooten, F.J. and van Delft, J.H.M. (2006) Modulation of gene expression and DNA adduct formation in HepG2 cells by polycyclic aromatic hydrocarbons with different carcinogenic potencies. *Carcinogenesis* 27, 646-655.

Staal, Y.C.M., van Herwijnen, H.M., Pushparajah, D., Umachandran, M., Ioannides, C., van Schooten, F.J. and van Delft, J.H.M. (2007) Modulation of gene expression and DNA adduct formation in precision-cut liver slices by polycyclic aromatic hydrocarbons with different carcinogenic potency. *Mutagenesis* 22, 55-62.

Steensma, A., Beaman, J.A., Walters, D.G., Price, R. J. and Lake, B. G. (1994) Metabolism of coumarin and 7-ethoxycoumarin by rat, mouse, guinea pig, *Cynomolgus* monkey and human precision-cut liver slices. *Xenobiotica* 24, 893-907.

Till, M., Riebniger, D., Schmitz, H.J. and Schrenk, D. (1999) Potency of various polycyclic aromatic hydrocarbons as inducers of CYP1A1 in rat hepatocyte cultures. *Chem.-Biol. Interact.* 117, 135-50.

Umachandran, M., Howarth, J. and Ioannides, C. (2004) Metabolic and structural viability of precision-cut rat and lung slices in culture. *Xenobiotica* 34, 771-780.

Umachandran, M. and Ioannides, C, (2006) Stability of cytochromes P450 and phase II conjugation systems in precision-cut lung slices cultured up to 72h. *Toxicology* 224, 14-21.

Vakharia, D.D., Liu, N., Pause, R., Fasco, M., Bessette, E., Zhang, Q.Y. and Kaminsky, L.S. (2001) Polycyclic aromatic hydrocarbon/metal mixtures: effect on PAH induction of CYP1A1 in human HEPG2 cells. *Drug Met. Disp.* 29, 999-1006.

Van de Kerkhof, E.G., de Graaf, A.M., de Jager, M.H., Meijer, D.K.F. and Groothuis, G.M.M. (2005) Characterization of rat small intestinal and colon precision-cut slices as an in vitro system for drug metabolism and induction studies. *Drug Met. Disp.* 33, 1613-1620.

Walker, N.J., Crofts, F.G., Li, Y., Lax, S.F., Hayes, C.L., Strickland, P.T., Lucier, G.W. and Sutter, T.R. (1998) Induction and localisation of cytochrome P450 1B1 (CYP1B1) protein in the livers of TCDD-treated rats: detection using polyclonal antibodies raised to histidine-tagged fusion proteins produced and purified from bacteria. *Carcinogenesis* 19, 395-402.

Whitlock, J.P. Jr. (1999). Induction of cytochrome P4501A1. *Ann. Rev. Pharmacol. Toxicol.* 39, 103-125.

Table 1: Taq Man® primers and probes for rat CYP1A1 and CYP1B1

The location is relative to position one of the accession number. Probes were labelled with 5' TAMRA and 3' FAM

Gene	Accession Number	Location	5' primer	3' primer	Probe
CYP1A1	NM_012540	97 –122	GCCTTCACATCAGC CACAGA	TTGTGACTCTAAC CACCCAGAATC	TGGCCGTCACCA CATTCTGCCTT
CYP1B1	NM_012940	2696 – 2775	TTCAGCTGTTCAAA CGAAGCA	TCCCAAAGTTGA AGCTTACGTTA	CGAGTTATGAGGGA GAAAAAGGTTTGCCA

Table 2: Ligand binding of the six PAHs to the Ah receptor

Binding of the PAHs to the Ah receptor was performed using the CALUX assay. EC₅₀ values were determined graphically from plots of substrate concentration (M) vs binding to the Ah receptor expressed in terms of TCDD binding (10⁻⁹ M).

Polycyclic aromatic hydrocarbon	Ah receptor binding affinity EC₅₀ (M)
Benzo[b]fluoranthene	5 x 10 ⁻⁸
Dibenzo[a,h]anthracene	5 x 10 ⁻⁷
Benzo[a]pyrene	5 x 10 ⁻⁶
Dibenzo[a,l]pyrene	15% at 10 ⁻⁴
Fluoranthene	13% at 10 ⁻⁴
1-Methylphenanthrene	No binding

Table 3: Structural and electronic characteristics of the six polycyclic aromatic hydrocarbons

L, Molecular length; W, Molecular width; D, Molecular depth; L/W, Ratio of molecular length to molecular width; a/d^2 , ratio of molecular area ($l \times w$) to the square of depth; μ , dipole moment; E_{HOMO} , Energy of the Highest Occupied Molecular Orbital; E_{LUMO} , Energy of the Lowest Unoccupied Molecular Orbital; ΔE , $E_{\text{HOMO}} - E_{\text{LUMO}}$; IP, fold-increase in the *O*-deethylation of ethoxyresorufin/ μmol of compound.

	L (Å)	W(Å)	D(Å)	a/d^2	L/W	μ (Debye)	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE	IP
Benzo[b]fluoranthene	13.642	9.541	3.200	12.709	1.430	0.606	-8.3824	-1.2520	7.1304	29.38
Benzo[a]pyrene	12.871	9.452	3.200	11.881	1.362	0.049	-7.6442	-1.3703	6.2739	14.79
Dibenzo[a,h]anthracene	13.970	9.458	3.200	12.903	1.477	0.002	-8.0476	-1.0143	7.0333	19.13
Dibenzo[a,l]pyrene	13.674	11.887	3.200	15.978	1.158	0.209	-7.7544	-1.3276	6.4267	1.80
Fluoranthene	11.302	9.541	3.200	10.531	1.185	0.511	-8.4298	-1.2244	7.2054	0.04
1-methylphenanthrene	11.569	7.930	4.201	5.198	1.459	0.298	-8.3702	-0.5555	7.8147	0.02

Legends to figures

Figure 1: Structure of PAHs used in the study

Figure 2: Concentration-dependent induction of ethoxyresorufin *O*-deethylase activity by PAHs in precision-cut rat liver slices.

Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations for 24 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and ethoxyresorufin *O*-deethylase (EROD) activity determined. Results are expressed as mean $\pm$ SD of triplicate pools of slices.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Benzo[a]pyrene data from Pushparajah *et al* (2007)

Figure 3: Concentration-dependent changes in CYP1A1 apoprotein levels in precision-cut rat liver slices incubated with PAHs.

Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations for 24 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and proteins resolved by 10% (w/s) SDS-PAGE before being transferred electrophoretically to nitrocellulose paper. Immunoblot was carried out with anti-rat CYP1A1 followed by peroxidase-labelled anti-goat IgG; all lanes were loaded with 5 μ g protein. Figures above the immunoblot represent percentage values compared with control (DMSO-treated) slices which were set at 100%.

Benzo[a]pyrene data from Pushparajah *et al* (2007)

Figure 4: Concentration-dependent changes in CYP1A1 mRNA levels in precision-cut rat liver slices incubated with PAHs.

Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations for 24 hours. At the end of the incubation period, slices were removed from the media and total RNA extracted. CYP1A1 mRNA levels were determined by quantitative RT-PCR (TaqMan). Changes are shown as fold-increases compared to control slices incubated with DMSO only. Results are expressed as mean $\pm$ SD where $n = 4$.

*** $P < 0.001$

Benzo[a]pyrene data from Pushparajah *et al* (2007)

Figure 5: Concentration-dependent changes in CYP1B1 mRNA levels in precision-cut rat liver slices incubated with PAHs.

Precision-cut rat liver slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations for 24 hours. At the end of the incubation period, slices were removed from the media and total RNA extracted. CYP1B1 mRNA levels were determined by quantitative RT-PCR (TaqMan). Changes are shown as fold-increases compared to control slices incubated with DMSO only. Results are expressed as mean $\pm$ SD where n=4.

* P<0.05; ** P<0.01; *** P<0.001

Benzo[a]pyrene data from Pushparajah et al (2007)

Figure 6: Concentration-dependent induction of ethoxyresorufin *O*-deethylase activity by PAHs in precision-cut rat lung slices.

Precision-cut rat lung slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations (0-5 μ M) for 48 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and ethoxyresorufin *O*-deethylase (EROD) activity determined. Results are expressed as mean $\pm$ SD of triplicate pools of slices.

* P<0.05; ** P<0.01; *** P<0.001

Benzo[a]pyrene data from Pushparajah et al (2007)

Figure 7: Concentration-dependent changes in CYP1A1 apoprotein levels in precision-cut rat lung slices incubated with PAHs.

Precision-cut rat lung slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations (0-5 μ M) for 48 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and proteins resolved by 10% (w/s) SDS-PAGE before being transferred electrophoretically to nitrocellulose paper. Immunoblot was carried out with anti-rat CYP1A1 followed by peroxidase-labelled anti-goat IgG; lanes with control slices were loaded with twice the amount of protein, except in the case of fluoranthene and 1-methylphenanthrene. Figures above the immunoblots represent percentage values, corrected for amount of protein loaded, compared with control (DMSO-treated) slices which were set at 100%.

Benzo[a]pyrene data from Pushparajah et al (2007)

Figure 8: Concentration-dependent changes in CYP1B1 apoprotein levels in precision-cut rat lung slices incubated with PAHs.

Precision-cut rat lung slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations (0-5 μ M) for 48 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and proteins resolved by 10% (w/s) SDS-PAGE before being transferred electrophoretically to nitrocellulose paper. Immunoblot was carried out with anti-rat

CYP1B1 followed by peroxidase-labelled anti-goat IgG; lanes with control slices were loaded with twice the amount of protein, except in the case of fluoranthene, dibenzo[a,l]pyrene and 1-methylphenanthrene. Figures above the immunoblots represent percentage values, corrected for amount of protein loaded, compared with control (DMSO-treated) slices which were set at 100%.

Benzo[a]pyrene data from Pushparajah et al (2007)

Figure 9: Metabolism of 7-ethoxycoumarin by precision-cut human liver slices from two different donors.

Precision-cut human liver slices from two donors were incubated with 7-ethoxycoumarin (50 μ M) for 6 hours, and metabolites were determined in the media. Results are presented as mean $\pm$ SD of triplicate slices.

Figure 10: Concentration-dependent induction of ethoxyresorufin *O*-deethylase activity by PAHs in precision-cut human liver slices.

Precision-cut human liver slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations (0-50 μ M) for 24 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and ethoxyresorufin *O*-deethylase (EROD) activity determined. Results are expressed as mean $\pm$ SD of triplicate pools of slices.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Figure 11: Concentration-dependent changes in CYP1A1 apoprotein levels in precision-cut human liver slices incubated with PAHs.

Precision-cut human liver slices were incubated in the absence and presence of the various PAHs, dissolved in DMSO, at a range of concentrations (0-50 μ M) for 24 hours. At the end of the incubation period, slices were removed from the media, microsomes prepared and proteins resolved by 10% (w/s) SDS-PAGE before being transferred electrophoretically to nitrocellulose paper. Immunoblot was carried out with anti-rat CYP1A1 followed by peroxidase-labelled anti-goat IgG; all lanes were loaded with 5 μ g protein. Figures above the immunoblot represent percentage values compared with control (DMSO-treated) slices which were set at 100%.

Figure 12: Effect of treatment with benzo[a]pyrene and β -naphthoflavone on rat hepatic and pulmonary CYP1B1 apoprotein levels.

Rats received intraperitoneally a single dose of either benzo[a]pyrene or β -naphthoflavone (25 mg/kg) and were killed 24 hours later. Solubilised hepatic and pulmonary microsomal proteins were resolved by 10% (w/s) SDS-PAGE before being

transferred electrophoretically to nitrocellulose paper. Immunoblot was carried out with anti-rat CYP1B1 followed by peroxidase-labelled anti-goat IgG; all lanes were loaded with 5 µg of protein. Figures above the immunoblots represent percentage values, corrected for amount of protein loaded, compared with control (DMSO-treated) slices which were set at 100%. Lanes 1 and 4, control liver and lung respectively; lanes 2 and 5, β-naphthoflavone liver and lung respectively; lanes 3 and 6, benzo[a]pyrene liver and lung respectively.

Figure 13: Binding of PAHs to the Ah receptor.

H1L1.1c2 cells were incubated with a range of concentrations of PAHs (10^{-12} M – 5×10^{-5} M), dissolved in DMSO, for 24 hours at 37°C and 5 % CO₂ in a humid environment. Binding to the receptor is expressed as % of TCDD binding (10^{-9} M). Results are presented as mean ± SD of triplicate determinations.

Figure 14: Correlation between induction of ethoxyresorufin *O*-deethylase activity by PAHs in precision-cut lung and liver slices.

Figure 15: Correlations between CYP1 induction potency of PAHs and molecular size.

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