Biochemical Pharmacology, Vol. 45, No. 7, pp. 1373–1386, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

TRANSPLACENTAL TRANSFER OF POLYCHLORINATED BIPHENYLS INDUCES SIMULTANEOUSLY THE EXPRESSION OF P450 ISOENZYMES AND THE PROTOONCOGENES c-Ha-ras AND c-raf

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(*Received* 4 *August* 1992; *accepted* 21 *January* 1993)

Abstract—At day 15 of gestation, rats were injected with a single i.p. dose of 100, 250 and 500 mg/kg body weight of a mixture of polychlorinated biphenyls (PCBs) (Aroclor 1254). Seven days later, significant increases in maternal and foetal cytochrome P450, cytochrome b_5 and cytochrome *c* (P450) reductase were found. Concomitantly, the metabolism of nitroanisole, aniline, ethoxyresorufin and benzo[*a*]pyrene was significantly increased, but foetal metabolism of dimethylnitrosamine was not detectable and only marginal increases in the metabolism of aminopyrine and aldrin were seen. In contrast, maternal metabolism of dimethylnitrosamine, aminopyrine and aldrin was measurable, but significant increases were determined only with the latter substrate. Transplacental transfer of PCBs resulted in increased metabolism of substrates catalysed by foetal CYP1A1 and CYP2B1, but there was no evidence for CYP2E1-catalysed reactions. Further measurements show significant increases in foetal and maternal epoxide hydrolase, glutathione-*S*-transferase and UDP-glucuronyl transferase activities, thus suggesting that treatment with Aroclor 1254 resulted in coordinated increases in foetal and maternal oxidative and post-oxidative drug metabolism. Western blot analysis of microsomal proteins shows the induction of foetal and maternal CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP3A1 and CYP4A1. In addition, increased expression of CYP2C6 was seen with the mother but not the foetus. Unlike the mother, foetal rats did not express CYP2E1 and the expression of the above-listed P450 isoenzymes was greater in the mother than the foetus. Northern blot analysis shows significant increases in maternal and foetal CYP1A1, CYP1A2 and CYP2B1 mRNA. An increased amount of CYP3A1 mRNA was only seen with the mother, but not the foetus. Treatment of mothers with Aroclor 1254 resulted in reduced CYP2A1, CYP2C7, CYP2E1 and CYP4A1 mRNA. Insignificant differences in the expression of foetal CYP2A1 and CYP4A1 mRNA were found, but *in utero* exposure to PCBs reduced the amounts of CYP2E1 mRNA and there was no foetal CYP2C7 mRNA transcript. Treatment with Aroclor 1254 increased the expression of the protooncogenes c-Ha-ras and c-raf in the mother and the foetus, but at varying intensities. Pregnancy itself was linked to an increased expression of these protooncogenes. erbA and erbB mRNA was not detected.

Polychlorinated biphenyls (PCBs§) are a group of chemicals that receive wide attention due to their widespread distribution within the ecosphere. The first report of PCBs accumulating in the environment dates back to the mid-sixties and since then more than 1000 investigations have been reported detailing their occurrence and accumulation in a wide range of inorganic and organic matrices. The accumulation of PCBs throughout the ecosystem is shown by their presence in samples of air, snow, and ice, and in a wide range of organisms including plankton, fish and marine and land mammals, including humans [1]. It has been estimated that about 31% of the 1.2 million tonnes of PCBs that have been produced are present in the environment [2]. The majority of PCB oils have been used in electrical equipment or other products that are still in use and improper disposal due to deposition in landfills has led to the potential of pollution for many years to come [2], and individual PCB isomers and congeners are commonly present in human blood, milk and other tissue samples such as biopsy material taken from tumours [3–5].

It is well documented that PCBs modulate and disturb normal cellular functions [1, 6, 7]. Depending on the experimental conditions, treatment dose and duration and the method of application, PCBs elicit a wide range of toxic reactions. Several excellent reviews are available which provide detailed information on all aspects of the toxic properties of PCBs including their ability to promote and perhaps to initate malignant tumour formation. It has been shown that mammalian species differ in their

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§ Abbreviations: PCB, polychlorinated biphenyl; GAPDH, glyceraldehyde phosphate dehydrogenase; EROD, ethoxyresorufin-*O*-deethylase; GST, glutathione-*S*-transferase.

response to PCBs and that developmental and sex differences are important factors to be considered in interpreting the toxicity of PCBs. Their potency in inducing certain P450 isoenzymes is well recognised and the studies by Parkinson *et al.* [8] show how versatile PCBs are in modulating the expression of these proteins.

Transplacental transfer of PCBs in humans has been reported and there is compelling evidence for *in utero* exposure to PCBs [9, 10]. Based on the evidence available to date, the risk for the mother and the foetus is significant, but it is notoriously difficult to find universal markers that allow risk assessment with high predictive power. Furthermore, as PCBs produce at the same time a variety of different toxic reactions it is difficult to define a suitable endpoint for a universal interpretation of the toxic properties of PCBs.

In the present investigation two markers were chosen as toxicological endpoints, namely, the expression of P450 isoenzymes and protooncogenes measured at the protein and mRNA level. Both endpoints have been shown to correlate with hepatocarcinogenesis [11]. The aim of the present study was therefore to assess early changes in the expression of P450 proteins from families 1–4 and to correlate these changes with substrate reactions catalysed by these isoenzymes. Furthermore, the concomitant expression of four different proto-oncogenes was studied to obtain detailed information on the simultaneous expression of the mRNA of P450 and protooncogenes. The result, taken collectively, should aid the search in defining suitable markers which predict the risk for the mother and the foetus, at early stages.

MATERIALS AND METHODS

Animals

Female Sprague–Dawley rats were supplied by Charles River Ltd (Margate, U.K.), with an average body weight of 175 g. Food and water was provided *ad lib*. The beginning of pregnancy was determined by examination of vaginal plugs.

Treatment of animals. At the 15th day of gestation rats were injected with a single i.p. dose of either 100, 250 or 500 mg/kg body weight Aroclord 1254 (a complex mixture of individual PCB isomers and congeners) dissolved in cornoil and killed 7 days later.

Foetal rats were surgically removed and the hepatic tissue was pooled. In some cases, natural delivery had occurred and offspring to the age of 12 hr post partum were included.

Chemicals, reagents, enzymic substrates

Unless otherwise stated, all chemicals and reagents were of highest purity and purchased from the Sigma Chemical Co. and BDH Chemicals (both Poole, U.K.).

NADPH-dependent enzyme reactions were assayed by the addition of 12 mM $NADP^+$, 12 mM glucose 6-phosphate and 60 U of glucose-6-phosphate dehydrogenase in a final volume of 3 mL. Cytochrome P450-catalysed reactions were assayed in a buffer system containing 0.1 M Tris, pH 7.4; 0.15 M $MgCl_2$ and 0.5 M nicotinamide to prevent the destruction of pyridine nucleotide by tissue nucleosidases.

Preparation of heptaic microsomes and assessment of microsomal and cytosolic enzyme activity

Hepatic microsomes were prepared as described previously [12]. Cytochrome P450 and b_5 were measured by the methods of Omura and Sato [13]. Protein concentrations were determined by the method of Lowry *et al.* [14] using bovine serum albumin as standard. Ethoxyresorufin-*O*-deethylase (EROD) activity was determined by the method of Burke *et al.* [15] and the N-demethylation of aminopyrine and dimethylnitrosamine was measured essentially as described by Anderson *et al.* [16]. Aldrinepoxidation was measured by the method of Wolff *et al.* [17] with modifications as described previously [18]. Activity of NADPH cytochrome *c* (P450) reductase, para-hydroxylation of aniline and O-demethylation of nitroanisole and of glucuronyl transferase using *o*-aminophenol as substrate were assayed as detailed in Ref. 19 with modifications described previously [18]. Glutathione-*S*-transferase (GST) activity was measured by the method of Habig *et al.* [20] using 2,4-dinitro-1-chlorobenzene as a substrate. Epoxide hydrolase was assayed using epichlorohydrin as a substrate and an experimental protocol detailed by Guengerich and Mason [21].

Unless otherwise stated, all enzyme kinetic measurements were found to be linear with respect to protein concentration and incubation time. A detailed description of the above described assays and the validation of linear enzyme kinetics is given by Borlakoglu [18].

Western immunoblot analysis of CYP isoenzymes

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to the method of Laemmli [22], using 9% separating gels. Immunoblotting was performed essentially as described by Towbin *et al.* [23] with modifications according to Lewis *et al.* [24]. After separation, proteins were transferred electrophoretically to nitrocellulose, and probed with various antisera to rat liver cytochrome P450s. Antibodies to the purified enzymes were isolated as described previously by Wolf *et al.* [25, 26]. These antibodies have been used previously in immunoblotting studies with mouse, rat and human microsomal samples. In addition, the isoenzyme specificity of the antisera has been demonstrated by immunoblot analysis which expressed human recombinant P450 proteins [27].

Cytochrome CYP4A1 was isolated according to the method of Tamburini *et al.* [28] and was of high purity, running as a single band on SDS–PAGE. Antibodies used were to CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C6, CYP2E1 (kindly provided by Dr C. W. Yang), CYP3A1 and CYP4A1 (distinct isoenzymes of the cytochrome P450 gene superfamily that catalyse oxidative reactions, for nomenclature system, see Nebert *et al.* [29]). A protein loading of 3 μg total protein/well was applied to enable direct comparisons amongst individual groups. Furthermore, the intensity of Coomassie blue staining of SDS–PAGE-separated proteins was

compared to ensure a uniform loading (data not shown).

After visualization of the immunoreactive polypeptides using horseradish peroxidase-labelled second antibody, the signal was enhanced with ^{125}I-protein A (Amersham International, Amersham, U.K.) and subsequent autoradiography (Kodak X-Omat AR5 X-ray film) at −70°. Differing exposure times were used in order to optimize the autoradiographic signal.

Northern blotting and mRNA determination of CYP isoenzymes

RNA was isolated from liver samples of female rats as described previously by Cox [30]. RNA concentration and purity were estimated spectrophotometrically and tested for integrity and equivalence of loading by ethidium bromide staining of a denaturing formaldehyde gel. mRNA was separated and P450 mRNA content determined on denaturing formaldehyde gels using hybridization conditions described previously [27]. Blots were washed at 65° with 0.3 M sodium chloride, 0.03 M trisodium citrate, pH 7.4. cDNA probes were labelled by the random-priming method [31–33]. Human cDNA probes used were CYP1A1, CYP2A6, CYP2B1, CYP2E1 (kind gift of Dr F. Gonzalez) and CYP3A3/4. The CYP2B1, CYP2C7 and CYP4A1 (kind gift of Dr G. Gibson; Earnshaw *et al.* [34]) cDNA probes were isolated from the rat.

Plasmids and cDNA probes used for northern blot hybridization of protooncogenes. The following DNA fragments were used: v-Ha-ras, 0.62 kb *Hin*dIII to *Bam*H1 fragment from the Balb-MuSV-DNA; v-erbA, 0.5 kb *Pst*I fragment of the AEV genome; v-erbB, 0.5 kb *Bam*HI fragment of the AEV genome; v-raf, 1.38 kb *Xho*I to *Bg*1II v-raf specific fragment of the 3611 MSV genome. Glyceraldehyde phosphate dehydrogenase (GAPDH) 1.1 kb PstI fragment of the GAPDH gene cloned in pBR322.

Further details about the plasmid and DNA probes were reported previously [35].

Northern blot hybridization of protooncogenes. Protooncogene mRNA content was determined following electrophoresis of RNA (10 μg/lane) with glyoxal in 1% agarose gels. Gels were stained with Acridine orange prior to the transfer of the RNA onto nitrocellulose membranes. RNA was subsequently fixed to the membranes by baking at 80° for 2 hr. RNA blots were prehybridized for about 3 hr at 42° in hybridization mixture containing 50% formamide, 5 × Denhardts (50 × Denhardts is 1% bovine serum albumin, 1% Ficoll, 1% polyvinylpyrollidone), 0.1% SDS, 5 × SSPE in 3.6 M NaCl, 0.2 M sodium phosphate, 0.02 M EDTA, pH 7.5) and 100 μg denatured salmon sperm DNA, pH 7.5. Hybridizations were carried out in the same buffer containing approx. 2×10^6 cpm/mL of ^{32}P-labelled DNA probes for 1 day at 42°. The DNA probes were ^{32}P-labelled by multiprime DNA labelling system (Amersham Buchler GmbH, D-3300 Braunschweig, Germany). After hybridization the filters were washed at 50° in 2 × Standard saline citrate, 0.1% SDS and exposed to Kodak X-Omat S film or Konica X-ray film A2 for desired periods. The relative abundance of transcripts was determined by densitometric analysis of the autoradiographs using an Elscript 400, Hirschmann (D-8025 Unteraching, Germany). It was confirmed that the scanning was done in the linear range of the film. Northern blots were rehybridized to GAPDH to adjust for uneven transfer during the blotting procedure or due to variations in the amount of mRNA applied to the gel. The expression of this gene serves as an internal control for mRNA quantitation.

RESULTS

The liver weights, body weights and microsomal protein concentrations are given in Table 1. Treatment with 250 mg/kg body weight Aroclor 1254 resulted in significant 3.2-fold and 3.9-fold increases in maternal and foetal microsomal protein concentrations, respectively. Increasing the treatment dose to 500 mg/kg body weight did not result in statistically significant increases in maternal microsomal protein concentrations. However, a highly significant 6.8-fold increase in foetal microsomal protein concentration was measured. This suggests a differential and dose-dependent effect, by which individual PCB isomers and congeners interfere with protein metabolism, but it should be pointed out that the number of animals entering the trial was small. The liver weight expressed as a percentage of the total body weight did not differ when control and Aroclor 1254-treated mothers were compared, but a significant ($P < 0.02$) reduction in

Table 1. The effects of Aroclor 1254 treatment on maternal and foetal liver weight, liver to body weight ratio and hepatic microsomal protein concentration

	Liver weight (g)		Microsomal protein (mg/g liver)		Liver to body weight ratio	
	Maternal	Foetal†	Maternal	Foetal†	Maternal	Foetal†
Control (N = 4)	17.07 ± 1.91	2.53 ± 1.04	16.4 ± 3.7	5.9 ± 1.8	5.59 ± 0.25	7.21 ± 0.47
250 mg/kg (N = 3)	17.32 ± 1.53	1.0 ± 0.19	53.6 ± 8.9*	23.2 ± 0.5*	5.26 ± 1.08	4.83 ± 0.88
500 mg/kg (N = 5)	19.27 ± 2.05	1.6 ± 0.38	33.7 ± 12.4	40.1 ± 5.6*	5.13 ± 0.76	6.23 ± 1.15

Values are means ± SD; N, number of animals; significantly different from the control *$P < 0.01$.
† Encompasses the entire litter with aliquots taken for individual measurements.

Table 2. The effects of Aroclor 1254 treatment on maternal and foetal hepatic microsomal monooxygenase components, cytochrome P450, cytochrome b_5 and cytochrome c (P450) reductase

	Maternal	Foetal‡
Total cytochrome P450 (nmol/g liver)		
Control (N = 4)	6.4 ± 0.5	1.3 ± 0.3
250 mg/kg (N = 3)	30.2 ± 0.8†	4.0 ± 0.5†
500 mg/kg (N = 5)	20.7 ± 1.1†§	6.0 ± 0.5†§
Cytochrome b_5 (nmol/g liver)		
Control (N = 4)	5.7 ± 0.3	0.8 ± 0.1
250 mg/kg (N = 3)	15.7 ± 1.8†	2.6 ± 0.3†
500 mg/kg (N = 5)	10.6 ± 1.8†§	1.5 ± 0.2†§
NADPH (P450) reductase (nmol cytochrome c reduced/g liver/min)		
Control (N = 4)	15 ± 0.6	10.8 ± 4.6
500 mg/kg (N = 5)	44 ± 0.6†	45 ± 4.6†

Values are means ±SD; N, number of animals; significantly different from the control *$P < 0.02$, †$P < 0.01$.

‡ Encompasses the entire litter with aliquots taken for individual measurements; §significantly different from the 250 mg/kg treatment dose.

the foetal liver to body weight ratio was noted at a treatment dose of 250 mg/kg body weight. The differences in this ratio were not significant when maternal rats were treated with 500 mg/kg body weight.

Effect of PCB treatment on microsomal monooxygenases

The result of cytochrome P450 and b_5 quantitation are summarized in Table 2. Control mothers had 4.9-fold and 7.3-fold higher concentrations, respectively, when compared with foetal rats. Treatment of pregnant rats with Aroclor 1254 (250 mg/kg) produced significant 4.7-fold and 2.7-fold increases in cytochrome P450 and cytochrome b_5, respectively, and 3-fold increases in foetal rats. The high dose of 500 mg/kg body weight did not yield further increases but a significant reduction ($P < 0.01$) in maternal cytochrome P450 and cytochrome b_5, when compared with the 250 mg/kg dose. In contrast, foetal cytochrome P450 was further increased ($P < 0.01$) at the higher treatment dose, but the expression of cytochrome b_5 was significantly ($P < 0.01$) reduced, when compared with the 250 mg/kg dose. In addition 2.9-fold and 4.1-fold increases in the activities of NADPH cytochrome c (P450) reductase were observed using maternal and foetal microsomal preparations, respectively, following treatment with Aroclor 1254 at 500 mg/kg.

Enzyme activities using marker substrates for CYP1A1 and CYP1A2

The catalytic activities of maternal and foetal cytochrome P450 were assessed and the results are shown in Table 3. Significant increases in the metabolism of all substrates were recorded in response to treatment with Aroclor 1254. The rates of EROD activity were increased 20-fold and 15-fold, respectively, when maternal and foetal microsomal preparations were used from PCB-treated animals (250 mg/kg body weight treatment dose). A further approximately 2-fold increase in EROD activities was noted, when pregnant rats were treated with a dose of 500 mg/kg body weight, but 38-fold and 16-fold increases in these monooxygenase activities were measured when the activities of maternal and foetal microsomal preparations were compared with values estimated for control animals.

Hepatic microsomes prepared from foetal controls did metabolize nitroanisole at a low but measurable rate, and the activities measured were only one third of the values determined for maternal rats. Treatment of pregnant rats with 500 mg/kg body weight Aroclor 1254 results in a highly significant 50-fold increase in the rates of foetal nitroanisole metabolism and it was found that the responsiveness of the foetus to PCB treatment was 7-fold greater, when compared with maternal (pregnant) rats. Hepatic microsomes prepared from foetal/perinatal hepatic tissue were apporoximately 2-fold more active in the O-demethylation of nitroanisole than microsomes prepared from pregnant rats.

Aniline is preferentially metabolized by CYP1A2, but there is also evidence for its metabolism by CYP2E1 [36]. The values reported in Table 3 are good estimates of the catalytic activities of foetal CYP1A2 as we were unable to measure the N-demethylation of dimethylnitrosamine (see Table 4 for details) which is a reaction specifically catalysed by CYP2E1. As shown in Table 3 foetal hepatic microsomes did not hydroxylate aniline, whereas the activities reported for maternal rats are in accordance with values published previously. Treatment of pregnant rats with Aroclor 1254 resulted in an approximate 6-fold increase in the metabolism of aniline but transplacental transfer of PCBs produced foetal activities accounting for less than 9% of maternal microsomal preparations. These results, taken collectively, show individual PCB isomers and congeners to modulate differentially the induction of CYP1A1 and CYP1A2 and comparable results are found for the foetus in response to *in utero* PCB exposure. [^{3}H]Benzo[*a*]pyrene was chosen as an additional substrate to measure the catalytic activities of the CYP1A subfamily. Further differences were obtained when the 3- and 9-hydroxylation of [^{3}H]-benzo[*a*]pyrene metabolism was assessed. These regioselective hydroxylation reactions are relatively specific to CYP1A1, as other CYPs are only marginally involved in the formation of these metabolites [36]. As detailed in Table 3, the 3- and 9-hydroxylation of [^{3}H]benzo[*a*]pyrene by maternal microsomes increased 9.5-fold and 30.9-fold, respectively, whereas a 1.9-fold and 5.1-fold increase was noted using foetal microsomal preparations. This suggests a differential responsiveness of maternal and foetal rats by which individual PCB isomers and congeners modulate the expression of CYP1A1.

Treatment of pregnant rats with Aroclor 1254 produced a highly significant 9-fold increase in the rate of total [^{3}H]benzo[*a*]pyrene metabolism whereas a 2-fold increase was measured with foetal preparations. Maternal animals were more respon-

Table 3. The effects of Aroclor 1254 treatment on enzyme reactions predominantly catalysed by maternal and foetal CYP1A1 and CYP1A2

	Maternal	Foetal‡
p-Hydroxylation of aniline (nmol/g liver/30 min)		
Control (N = 3)	147 ± 3	not detectable
500 mg/kg (N = 5)	924 ± 21†	18 ± 2
Nitroanisole-O-demethylation (nmol/g liver/15 min)		
Control (N = 4)	10 ± 2	3 ± 1
500 mg/kg (N = 5)	73 ± 3*	158 ± 9†
EROD activity (nmol/g liver/min)		
Control (N = 4)	0.3 ± 0.1	0.2 ± 0.1
100 mg/kg (N = 3)	4.8 ± 0.2*	ND
250 mg/kg (N = 3)	6.4 ± 0.3†	2.5 ± 0.4
500 mg/kg (N = 3)	12.1 ± 1.1†	2.8 ± 0.4
AHH activity (pmol/mg protein/min)		
Control (N = 3)	112 ± 20	64 ± 1
250 mg/kg (N = 3)	1032 ± 17†	126 ± 6†
Metabolism of [^{3}H]benzo[*a*]pyrene to 3-OH and 9-OH phenols		
3-OH metabolite (control, N = 3)	34§	21§
3-OH metabolite (250 mg/kg, N = 3)	324§	40§
9-OH metabolite (control, N = 3)	2§	3§
9-OH metabolite (250 mg/kg, N = 3)	69§	15§

Values are means ± SD; N, number of animals; significantly different from the control *$P < 0.02$, †$P < 0.01$.

‡ Encompasses the entire litter with aliquots taken for individual measurements;

§ mean of pooled samples for the detection of individual metabolites.

ND, not determined.

AHH, aryl hydrocarbon hydroxylase.

Table 4. The effects of Aroclor 1254 treatment on enzyme reactions predominantly catalysed by maternal and foetal CYP2B1 and CYP2E1

	Maternal	Foetal†
N-Demethylation of dimethylnitrosamine (nmol/mg protein/min)		
Control (N = 4)	1.4 ± 0.2	not detected
500 mg/kg (N = 5)	11.3 ± 0.4	not detected
N-Demethylation of aminopyrine (nmol/mg protein/min)		
Control (N = 4)	1.2 ± 0.2	not detected
500 mg/kg (N = 5)	4.6 ± 0.3*	0.2 ± 0.1
Aldrin epoxidation (nmol/mg protein/min)		
Control (N = 3)	2.2 ± 0.6	3.2 ± 0.7
250 mg/kg (N = 5)	4.4 ± 0.9*	4.3 ± 1.1
500 mg/kg (N = 3)	4.1 ± 0.5*	3.6 ± 1.1

Values are means ± SD; N, number of animals; significantly different from the control *$P < 0.01$.

Encompasses the entire litter with aliquots taken for individual measurements.

sive to Aroclor 1254 treatment than foetal rats. A similar result was obtained when the rates of aniline metabolism by maternal and foetal microsomes were compared. Developmental differences in the expression and regulation of CYP1A1 may account for the greater responsiveness of maternal rats to PCB treatment.

Enzyme activities of marker substrates for the CYP2 family

The results of metabolism of marker substrates for the CYP2 family are summarized in Table 4 and show that treatment of maternal rats with Aroclor 1254 resulted in significant increases in the rates of aminopyrine N-demethylation and microsomal aldrin epoxidation. The increase in microsomal aldrin epoxidation was approximately 2-fold for both treatment doses but there was no evidence for an increase in foetal aldrin epoxidation. Studies by Parkinson *et al.* [37] and in our laboratory have shown a time-dependent induction profile with maximally expressed activities around 48 hr post-treatment and a subsequent decline in activities [18]. Therefore, the measurements detailed in Table 4 do not reflect the activities of maximally induced CYP2B1 as these measurements were carried out 7 days post-treatment. The other substrate which is to a large extent metabolized by CYP2B1, aminopyrine, was not demethylated at detectable rates by foetal hepatic microsomes of control animals, but transplacental transfer of PCBs resulted in a rate of metabolism which accounted for approximately 16% of the values estimated for maternal rats. Maternal rats responded to Aroclor 1254 treatment with 4-fold increases in aminopyrine metabolism. This increase was 2-fold greater when compared with the aldrin epoxidation data. Treatment of pregnant rats did not induce CYP2E1 as judged by the rates of N-demethylation of dimethylnitrosamine. This agrees with previous reports [18] but the metabolism of aminopyrine was significantly increased as shown in Table 4. However, the single time point measurements reported in Table 4 do not mirror the time-dependent profile in the induction of CYP2B1 and CYP2E1. The transient induction of these cytochromes contrasts with the induction of CYP1A1,

Table 5. The effects of Aroclor 1254 treatment on the activities of the Phase II enzymes, epoxide hydrolase, GST and UDP-glucuronyl transferase

	Maternal	Foetal‖
Epoxide hydrolase activity (nmol/g liver/min)		
Control (N = 4)	157 ± 17	288 ± 28
250 mg/kg (N = 5)	858 ± 150*	538 ± 53*
GST activity (nmol/mg protein/min)		
Control (N = 4)	111 ± 9	64 ± 8
100 mg/kg (N = 3)	292 ± 41*	106 ± 19*
250 mg/kg (N = 3)	230 ± 77*	136 ± 25*
500 mg/kg (N = 4)	376 ± 31*†	133 ± 32*
UDP-glucuronyl transferase activity (nmol/g liver/30 min)		
Control (N = 4)	130 ± 16	276 ± 72
100 mg/kg (N = 3)	1101 ± 217*	2208 ± 44*
250 mg/kg (N = 5)	2342 ± 430*‡	2179 ± 95*
500 mg/kg (N = 4)	3757 ± 298*‡§	592 ± 11*§

Values are means ± SD; N, number of animals; significantly different from the control *$P < 0.01$.

† Significantly different ($P < 0.05$) from the 250 mg treatment dose.

‡ Significantly different ($P < 0.01$) from the 100 mg treatment dose.

§ Significantly different ($P < 0.01$) from the 100 and 250 mg treatment doses.

‖ Encompasses the entire litter with aliquots taken for individual measurements.

as this isoenzyme is induced for a time period of up to several weeks. Foetal hepatic microsomes prepared from control rats did not metabolize dimethylnitrosamine and transplacental transfer of PCB isomers and congeners did not result in the expression of CYP2E1 (see below, western immunoblot analysis). This points to a complex regulation in the expression of foetal and maternal isoenzymes that are selectively induced by PCBs.

Effect of Aroclor 1254 *on the regulation of post-oxidative drug-metabolizing enzymes*

The results of post-oxidative drug metabolism reactions are summarized in Table 5 and show that treatment of pregnant rats with Aroclor 1254 caused a significant increase in GST activities. The activities determined for control foetal rats were 58% those for adult rats. Maternal GST activities did not increase in a dose-dependent fashion. However, a small 1.6-fold increase ($P < 0.05$) was recorded when the 500 mg/kg dose was compared with the 250 mg/kg dose. Transplacental transfer of PCBs resulted in an up to 2-fold increase in foetal GST activities but not in a dose-dependent fashion. These results may suggest at the lower dose a maximal induction of foetal and maternal glutathione *S*-transferase.

The conjugation of *o*-aminophenol by UDP-glucuronyl transferases was 2-fold more active with control foetal microsomal preparations when compared with maternal rats. Treatment of pregnant rats with Aroclor 1254 (see Table 5) resulted in highly significant dose-dependent 8-fold, 18-fold and 29-fold increases at 100, 250 and 500 mg/kg, respectively, in rates of *o*-aminophenol conjugation. The increases in the rates of conjugation were significantly different when individual treatment groups were compared as shown in Table 5. Transplacental transfer of PCB isomers and congeners resulted in an approximate 8-fold increase in foetal activities but there was no further increase when maternal rats received 250 mg/kg body weight Aroclor 1254. Indeed, treatment of maternal rats with 500 mg/kg body weight Aroclor 1254 produced a mere 2-fold increase in foetal UDP-glucuronyl transferase activities and this reduced responsiveness to an increased maternal treatment dose of Aroclor 1254 suggest a differential responsiveness of the mother and foetus in the expression of UDP-glucuronyl transferases.

Foetal rats expressed microsomal epoxide hydrolase activities approximately twice those of maternal rats. This suggests a differential regulation of epoxide hydrolase (Table 5) in foetal and pregnant rats. A highly significant 5.5-fold increase in activities was noted in response to maternal PCB treatment whereas a mere 1.9-fold increase was seen with foetal hepatic microsomes. Foetal animals appear to be less responsive to an induction by PCBs when assessed for epoxide hydrolase using epichlorohydrin as a substrate. That certain PCB isomers and congeners are potent inducers of epoxide hydrolase has been shown by Parkinson *et al.* [8].

Western blot analysis of proteins from P450 families 1–4

In addition to the enzymic measurements, western immunoblotting experiments were carried out to obtain unequivocal evidence on the expression of individual P450 isoenzymes present in maternal and foetal hepatic tissue. Furthermore, the expression of immunoreactive proteins was correlated with mRNA levels determined by northern blot analysis. Representative proteins of P450 families 1–4, i.e. CYP1A1, CYP1A2, CYP2B1, CYP2C6, CYP2E1, CYP3A1 and CYP4A1 were chosen as standards.

The expression of individual isoenzymes was determined by comparing immunoreactive bands and their electrophoretic mobility with those estimated for purified isoenzymes (i.e. standards). This comparison permitted an identification of constitutively expressed isoenzymes and the effects of PCB treatment on the induction of P450 proteins. The results of the western immunoblotting studies are summarized in Fig. 1. Control mothers had a strong immunoreactive band corresponding to CYP2C6 and a weak immunoreactive band corresponding to CYP3A1, but there was little evidence for the expression of CYP1A1, CYP1A2, CYP2B1, CYP2E1 and CYP4A1 at a loading rate of 3 μg total protein/well. A comparable result was obtained with control foetal hepatic microsomes (Fig. 1), but the expression of foetal CYP2C6 and CYP3A1 was weaker.

Treatment of pregnant rats with Aroclor 1254

The results of P450 protein induction in response to PCB treatment are summarized in Fig. 1. Comparison of control and PCB-treated mothers shows an impressive induction of CYP1A1, CYP1A2, CYP2A1, CYP2B1, CYP2C6, CYP3A1 and CYP4A1. This suggests that with the exception of

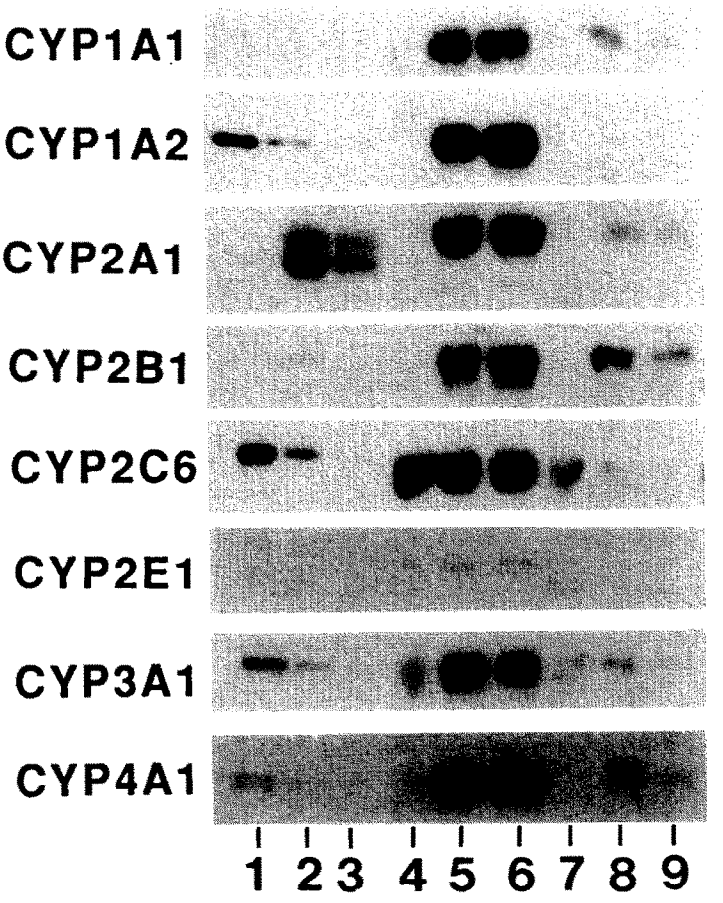

Fig. 1. Western blot analysis of maternal and foetal microsomal P450 proteins. Microsomal proteins were separated on SDS–PAGE, transferred to nitrocellulose and probed with anti-P450 antisera, as detailed in Materials and Methods. Lanes 1–3, standards (1.2, 0.8 and 0.4 pmol purified P450, respectively); lane 4, control mother; lanes 5 and 6, Aroclor 1254-treated mothers (100 and 250 mg/kg body weight, respectively); lane 7, control foetus; lanes 8 and 9, transplacental exposure to PCBs in response to a maternal treatment dose of 100 and 250 mg/kg body weight Aroclor 1254, respectively.

CYP2E1, Aroclor 1254 treatment of pregnant rats leads to induction of a wide range of P450 isoenzymes. This inductive response was, however, not uniform, as CYP2E1 was only marginally induced and the expression of CYP1A1 and CYP2B1 was less pronounced (Fig. 1). There was little evidence to suggest a dose-dependent expression of P450 isoenzymes. Therefore, the lower dose of 100 mg/kg body weight may represent a "saturation" response with respect to P450 induction. It is noteworthy that an increase in Aroclor 1254 treatment dose resulted in a reduction in CYP1A1 immunoreactive protein. This decline in immunoreactive protein is not properly understood, but other investigators have reported a similar decline in unidentified P450 isoenzymes in response to treatment with Aroclor 1254 [37].

Induction of foetal hepatic cytochrome P450 in response to the transplacental transfer of PCB isomers and congeners

The results of western immunoblotting of foetal hepatic microsomal proteins in response to transplacental transfer of PCBs are summarized in Fig. 1. When maternal rats and their foetal offspring were compared significant differences were observed. Antibodies raised to proteins encoded by the genes for CYP1A1, CYP2A1, CYP2B1, CYP3A1 and CYP4A1 cross-reacted with foetal hepatic microsomes. There was little expression of foetal CYP1A2 which contrasts with the mother, but agrees with the low rates of foetal para-hydroxylation of aniline reported in Table 3. The expression of foetal CYP1A1, however, points to a developmental regulation of the two genes of this subfamily (see northern blot analysis).

In addition, there are further dose-related effects. At the higher treatment dose, very significant reductions in the expression of foetal P450 proteins were observed. The most dramatic decline was seen with CYP1A2, CYP2C6 and CYP3A1 proteins where expression was lost at the highest PCB dose. In a similar fashion, the expression of CYP1A1, CYP2A1 and CYP4A1 proteins was reduced at the higher treatment dose.

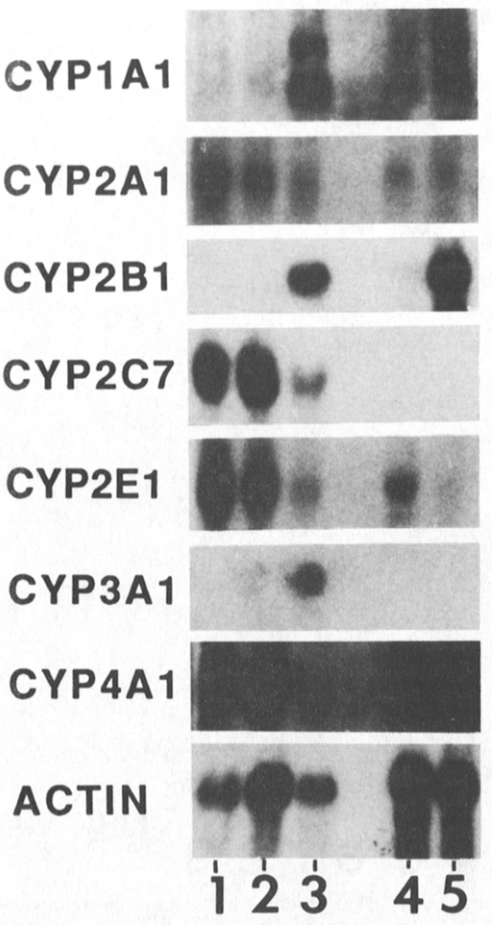

Fig. 2. Cytochrome P450 mRNA levels in maternal and foetal liver samples. RNA (10 μg) of maternal and foetal liver samples was separated on denaturing formaldehyde agarose gels, transferred to Hybond N and probed with P450 cDNA probes as outlined in Materials and Methods. Lane 1, control female; lane 2, pregnant mother; lane 3, Aroclor 1254-treated mothers (250 mg/kg body weight); lane 4, control foetus; lane 5, foetus, transplacentally exposed to PCBs.

Northern blot hybridization analysis of cytochrome P450 mRNA

Figure 2 summarizes the northern blot hybridization experiments. cDNAs were hybridized to CYP1A1, CYP1A2, CYP2A6, CYP2B1, CYP2C7, CYP2E1, CYP3A3/4 and CYP4A1 mRNA extracted from hepatic tissue of control and PCB-treated, pregnant and foetal rats. The molecular size of the mRNA transcripts and the densitometric analysis are detailed in Tables 6 and 7. It should be noted, that the CYP1A probe hybridized to two mRNA transcripts. The upper band (2.9 kb) was assigned to CYP1A2 whereas CYP1A1 was assigned to the lower band (2.2 kb). The molecular sizes of the individual mRNAs are detailed in Tables 6 and 7 and are consistent with the values reported by others [38]. For checking loading equivalence, a rat liver Actin probe was employed.

There was little expression of CYP1A1 and CYP1A2 mRNA in control pregnant rats, but treatment with Aroclor 1254 produced approximately 32-fold and 63-fold increases, respectively. With CYP2B1 and CYP3A1 mRNA, however, treatment with Aroclor 1254 produced approximately 39-fold and 4-fold increases, respectively, in mRNA levels. In contrast, treatment of pregnant rats with Aroclor 1254 produced a highly significant reduction in CYP2A1 mRNA and similar results were obtained when total RNA samples were probed for CYP2C7, CYP2E1 and CYP4A1 mRNA (Fig. 2). The results are reported in Table 6 and show that treatment with PCBs produces reductions to 15%, 25%, 27% and 25%, respectively, of control values. These reductions were somewhat unexpected as western blot analysis shows significant increases in CYP2A1, CYP3A1 and CYP4A1 immunoreactive proteins. The increases in CYP2A1, CYP3A1 and CYP4A1 proteins may be the result of protein stabilization. Such a mechanism of protein induction has been reported for CYP2E1 using pulse labelling with amino acids (reviewed in Ref. 38).

The northern blots are shown in Fig. 2, and densitometric scanning of the autoradiographs is given in Table 7. There was little expression of CYP1A1, CYP1A2, CYP2A6, CYP2B1 and CYP3A1 mRNA and there was no evidence for a CYP2C7 mRNA transcript. However, CYP2E1 and CYP4A1 mRNA transcripts were found in extracts of control foetal hepatic tissue.

Transplacental transfer of PCBs resulted in profound increases in CYP1A1, CYP1A2 and CYP2B1 mRNA. In contrast, exposure to PCBs caused a highly significant reduction in CYP2E1 mRNA. The northern blot analysis accords, in part, with the western blot analysis shown in Fig. 1, as the protein expression of CYP1A1 and CYP2B1 (Fig. 1) was paralleled by concomitant 6.8-fold and 16-fold increases in CYP1A1 and CYP2B1 mRNA.

Hybridization analysis of CYP1A1 and CYP1A2 mRNA (Fig. 2) details the presence of two transcripts as shown in Fig. 2. Densitometric scanning (Table 7) of northern blot autoradiographs suggests higher CYP1A2 mRNA levels in response to transplacental transfer of PCBs. In addition, a CYP4A1 mRNA transcript was found in control foetal rats, but a CYP4A1 cross-reactive protein was not expressed (Fig. 1). The expression of CYP4A1 was dependent upon Aroclor 1254 treatment and its expression was significantly reduced at the higher treatment dose (see also Discussion). Further evidence for the potential involvement of translation factors that are modulated by PCBs are detailed in Fig. 2. Foetal controls express CYP2E1 mRNA, but translation into protein was not found (Fig. 1). Treatment with PCBs significantly reduced the expression of CYP2E1 mRNA (Fig. 2, Table 7) and as shown in Fig. 1 there was no evidence for a cross-reactive protein. Furthermore, foetal controls did not express CYP2C7 mRNA and treatment of mothers with Aroclor 1254 did not modulate the precocious expression, as its activation is not apparent until puberty (reviewed in Ref. 38).

Expression of the cellular protooncogenes c-raf and c-Ha-ras, but not c-erbA and c-erbB

The results of northern blot hybridization

Table 6. Densitometric analysis of P450 mRNA levels in liver tissue of control and pregnant rats in response to treatment with 250 mg/kg body weight Aroclor 1254

Isoenzyme	Control pregnant	PCB-treated pregnant	*n*-fold change	Molecular size of cDNA probe (kb)
CYP1A1	166	5378	32.40	2.2
CYP1A2	166	10,458	63	2.9
CYP2B1	191	7506	39.30	2.0
CYP2C7	20,966	5405	0.26	1.8
CYP2E1	6320	1752	0.28	2.0
CYP2A1	1927	299	0.16	1.8
CYP3A1	1912	7910	4.13	1.8
CYP4A1	10,166	2586	0.25	2.1

Table 7. Densitometric analysis of P450 mRNA levels in liver tissue of control and foetal/perinatal rats exposed to a maternal treatment dose of 250 mg/kg body weight Aroclor 1254

Isoenzyme	Control foetus	PCB-exposed foetus	*n*-fold change	Molecular size of cDNA probe (kb)
CYP1A1	81	551	6.8	2.2
CYP1A2	81	1620	20	2.9
CYP2B1	551	9057	16	2.0
CYP2C7	10	15	—	1.8
CYP2E1	373	137	0.36	2.0
CYP2A1	753	190	0.25	1.8
CYP3A1	136	187	1.37	1.8
CYP4A1	5486	2875	0.52	2.1

experiments are summarized in Figs. 3 and 4. Considering c-raf (Fig. 3) expression, pregnancy causes an approximate 3-fold increase in c-raf mRNA. Pregnant and foetal rats expressed c-raf at similar levels. Treatment of pregnant rats with Aroclor 1254 caused a further 2-fold increase in c-raf mRNA, but hardly any increase was seen with *in utero* exposed foetal rats.

The changes in c-Ha-ras expression (Fig. 4) were less significant. Pregnancy itself was linked to an approximate 2-fold increase in c-Ha-ras expression. Foetal controls showed marginally less mRNA than that seen with the mother. Treatment of maternal rats failed to raise a further expression of this protooncogene. In contrast, foetal rats exposed to Aroclor 1254 showed a 2-fold greater expression of c-Ha-ras mRNA than controls.

DISCUSSION

Aroclor 1254 treatment of pregnant rats resulted in a significant induction of P450 isoenzymes. This inductive response was, however, not uniform and depended on the isoenzymes studied. There are differences in the inductive response, when maternal rats and their foetal offspring are compared, as the expression of foetal P450 isoenzymes is under developmental control.

Our results agree with the western blot data reported by Yang *et al.* [39] as foetal controls did not cross-react with antibodies raised to electrophoretically homogenous CYP1A1 and CYP1A2 (see Fig. 1). In addition, we are in agreement with the results reported by Giachelli and Omiecinski [40] as total RNA extracts of foetal controls failed to hybridize significantly with cDNA probes specific for CYP1A1 and CYP1A2 mRNA (see Fig. 2). However, *in utero* exposure of the foetus to PCB isomers and congeners resulted in an impressive CYP1A1 induction and therefore the present study provides additional evidence for its inducibility during foetal life. These results are in agreement with those reported by Yang *et al.* [39], where induction of foetal hepatic CYP1A1 was noted in response to a single dose of 40 mg/kg body weight of 3-methylcholanthrene given intraperitoneally to the mother 48 hr prior to killing. It is noteworthy that induction of foetal CYP1A1 can be achieved by treatment of mothers with either 3-MC or PCBs.

The results shown in Fig. 2 signify a large accumulation of CYP1A1 and CYP1A2 mRNA in response to transplacental transfer of PCBs. This contrasts with the observations reported by Giachelli and Omiecinski [40] as these authors failed to detect accumulation of foetal hepatic CYP1A1 and CYP1A2 mRNA in response to 3-MC treatment prior to 1 week after birth. The reasons for these differences are not apparent. However, the mode of CYP1A1/CYP1A2 induction by PCB isomers and congeners is comparable with that of 3-MC, since binding of these chemicals to the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin receptor is a prerequisite [38]. Other factors

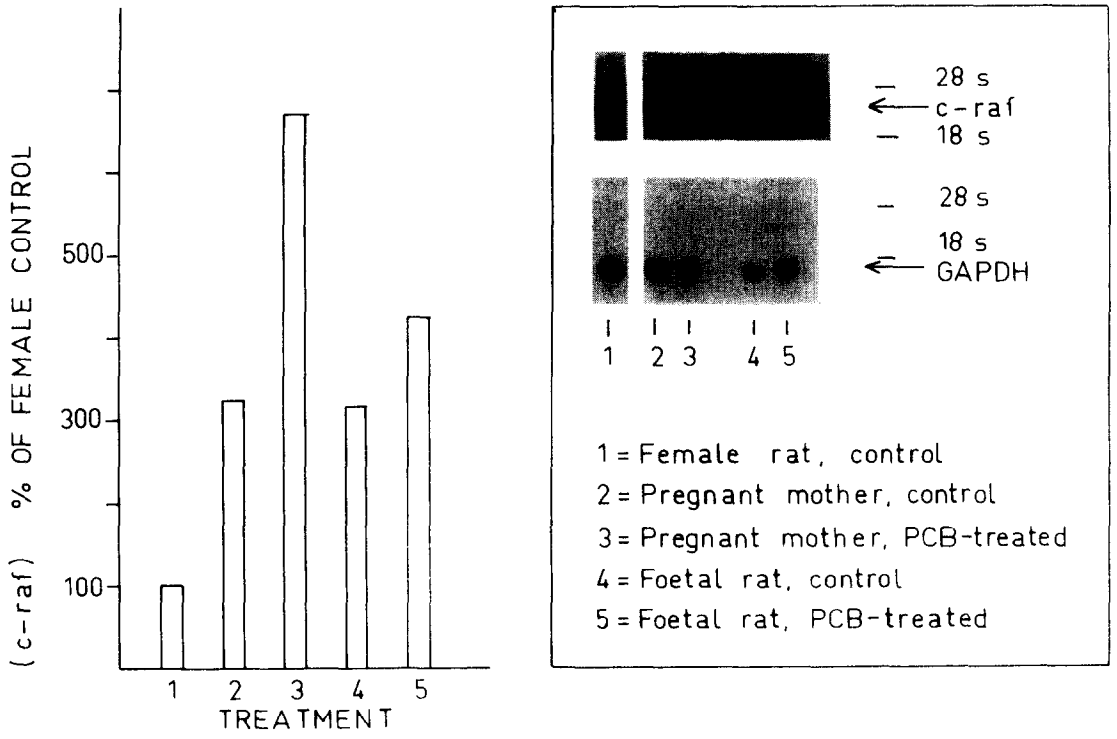

Fig. 3. c-raf mRNA levels in control and Aroclor-treated maternal and foetal rat liver samples. RNA from livers of control females, pregnant mothers and foetal rats was isolated and separated on agarose gels and transferred to nitrocellulose membranes. Ten micrograms of total RNA were probed with cDNA probes as outlined in Materials and Methods. The autoradiographic signals were quantified densitometrically and normalized to GAPDH mRNA.

such as differences in the half-lives of mRNAs in response to either 3-MC or PCB treatment (i.e. differences in the rate of mRNA degradation) may account for these contrasting results. The increased metabolism of the marker substrates EROD, *p*-nitroanisole and benzo[*a*]pyrene (see Table 3) provide additional evidence for the induction of CYP1A1.

As shown in Fig. 2, there was significant CYP1A2 mRNA accumulation in foetal hepatic tissue in response to transplacental transfer of PCB isomers and congeners, but there was little expression of CYP1A2 (see Fig. 1). Similar results were obtained when total RNA extract were probed for CYP2E1 mRNA and CYP4A1 mRNA (for discussion, see below). This indicates that PCBs may interfere with the translation of certain mRNAs. For comparison, the metabolism of aniline was not detectable in control foetal rats as they did not express CYP1A2 (see Table 3). However, treatment of mothers with Aroclor 1254 resulted in foetal metabolism of this substrate. It is noteworthy that aniline is additionally metabolized by CYP2E1, but western blot analysis did not reveal CYP2E1 protein expression (see Fig. 1). Therefore, the results reported in Table 3 are approximate values for the catalytic activities of foetal CYP1A2. Our results thus provide evidence at the mRNA, protein and enzyme catalytic level that CYP1A1 is inducible in foetal rats.

The induction of maternal CYP2A1 in response to treatment with Aroclor 1254 (see Fig. 1) agrees with the results reported by Parkinson *et al.* [8]. These authors have shown that treatment of 3-week-old Long Evan rats with Aroclor 1254 (500 mg/kg body weight) resulted in a 3–5-fold increase in immunoreactive protein. Treatment of male rats with single PCB isomers and congeners, namely, 3,4,5,3′,4′-penta- and 3,4,5,3′,4′,5′-hexachlorobiphenyl caused an unprecedented high level of induction. A similar result was obtained when male rats were treated with the brominated analogues 3,4,5,4′-tetra-, 3,3′,4,4′-tetra- and 3,4,5,3′,4′-pentabromobiphenyl. The results reported by Parkinson *et al.* [8] document how versatile PCBs are in modulating the expression of CYP2A1, as treatment with eight isomeric and congeneric mono-ortho-substituted halogenated biphenyls produced additional expression of this protein, albeit, at varying degrees.

In the present study, the induction of maternal and foetal CYP2A1 by Aroclor 1254 was not paralleled by an increase in cDNA hybridized CYP2A1 mRNA. Indeed, treatment of the mother with Aroclor 1254 produced a highly significant reduction to 15% of control values (see Table 6). Similarly, the level of foetal CYP2A1 mRNA showed a reduction to 25% of control values.

As discussed above, it is conceivable that treatment

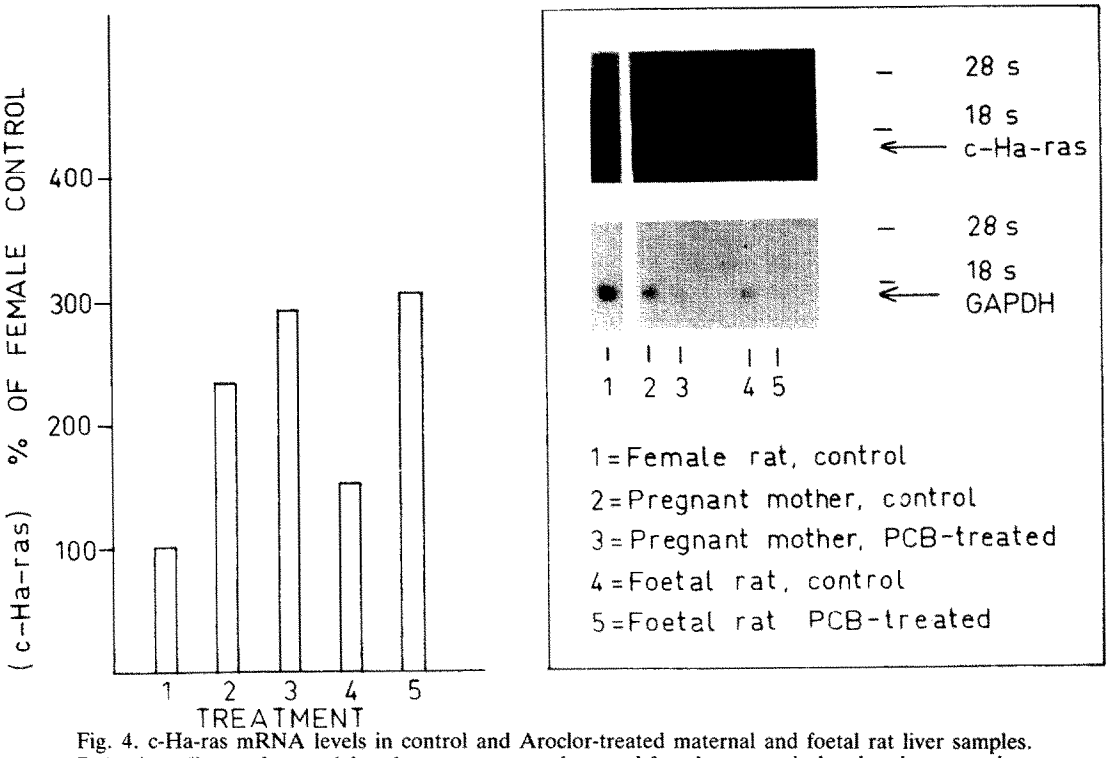

Fig. 4. c-Ha-ras mRNA levels in control and Aroclor-treated maternal and foetal rat liver samples. RNA from livers of control females, pregnant mothers and foetal rats was isolated and separated on agarose gels and transferred to nitrocellulose membranes. Ten micrograms of total RNA were probed with cDNA probes as outlined in Materials and Methods. The autoradiographic signals were quantified densitometrically and normalized to GAPDH mRNA.

with Aroclor 1254 resulted in an enhanced degradation of mRNA, but at the same time PCB isomers and congeners may bind to the P450 protein thereby reducing its rate of degradation. This could explain the apparent P450 induction shown in Fig. 1 despite the reduction in mRNA. Furthermore, control foetal microsomal proteins did not cross-react with the antibody raised to CYP2A1 (Fig. 1) which agrees with reports by others [38]. Immunoblotting shows the induction of foetal CYP2A1 in response to transplacental transfer of PCB isomers and congeners, but there was a reduction in immunoreactive protein at the higher treatment dose. This reduction was not observed with the mother, which suggests differences in the responsiveness of the mother and the foetus to its modulation by PCBs. Other workers [8, 37] have noted the suppression of unidentified forms of cytochrome P450 in response to Aroclor 1254 treatment as part of a pleiotropic response to treatment with halogenated biphenyls.

To date, it remains controversial whether transplacental transfer of phenobarbital induces the accumulation of CYP2B1 and CYP2B2 mRNA as studies have not shown a mRNA transcript prior to day 21 of gestation [40]. In contrast, other investigators reported the detection of total CYP2B1/2B2 mRNA in control mice and rats as early as day 17 of gestation [41]. Our results are in agreement with the above-cited reports as we were unable to detect CYP2B1 mRNA in control maternal and foetal hepatic tissue (Fig. 2 and Tables 6 and 7) which agrees with the above cited reports. However, treatment with Aroclor 1254 produced highly significant increases in maternal and foetal CYP2B1 mRNA (Fig. 2) and protein (Fig. 1) showing that transplacental transfer of PCBs evokes the expression of foetal CYP2B1.

The western blot analysis shows significant expression of CYP2C6 in control and Aroclor 1254-treated maternal rats (Fig. 1). PCB treatment did not produce an increased expression of this protein but transplacental transfer caused a suppression of CYP2C6 in foetal liver. The results may indicate enhanced degradation of foetal CYP2C6 in response to transplacental transfer of PCB isomers and congeners, but we also consider accelerated CYP2C6 mRNA degradation to be an alternative explanation. For comparison, the maternal CYP2C7 mRNA transcript is shown in Fig. 2 and a reduction of 25% of control values was found in response to Aroclor 1254 treatment. This may indicate enhanced degradation or reduced synthesis of CYP2C7 mRNA. The physiological implications of a diminished CYP2C7 mRNA accumulation in maternal liver in response to PCBs are uncertain. Transplacental transfer of PCBs did not, however, modulate the expression of foetal CYP2C7 mRNA.

Treatment of mothers with Aroclor 1254 did not induce the metabolism of *N*-nitrosodimethylamine

(Table 4), but western blot analysis shows a marginal increase in the expression of CYP2E1. Furthermore, northern blot analysis (Fig. 2) shows a reduction in CYP2E1 mRNA to 27% of control values. These results are contrasting, but nevertheless, are in agreement with reports by others (reviewed in Ref. 38). Indeed, CYP2E1 induction has been shown to occur in rats in response to treatment with acetone, pyrazole and ethanol without measurable increases in CYP2E1 mRNA. Recent experimental evidence [38, 42] suggests high affinity binding of certain inducers to the CYP2E1 protein to stabilize the protein against degradation. A similar binding of certain PCB isomers and congeners to CYP2E1 may stabilize the protein against degradation. For comparison, treatment of first day lactating rats with Aroclor 1254 (Borlakoglu JT, Scott A, Henderson C and Wolf CR, unpublished) caused significant CYP2E1 induction in the mother and the suckling. In addition, significant increases in maternal and neonatal CYP2E1 mRNA transcripts were found and concomitantly the metabolism of maternal and neonatal *N*-nitrosodimethylamine was increased. These results suggest a differential response to PCBs during pregnancy and lactation.

It is noteworthy that CYP2E1 was not detectable in foetal hepatic tissue and neither control nor PCB-exposed foetal rats metabolized *N*-nitroso-dimethylamine at detectable rates. Similar to maternal rats, transplacental transfer of PCB isomers and congeners produced a reduction of the CYP2E1 mRNA to 36% of control values. In control pregnant rats CYP2E1 was not detectable, but a mRNA transcript was found (Fig. 2). A comparable result was obtained for untreated perinatal rats. The reasons for a reduction in mRNA are unknown.

Investigations into the antenatal induction of CYP3A1 have been equivocal, as phenobarbital treatment of rats did not cause an induction until 15 days after birth (Cresteil *et al.* [43]). In contrast, in the same experiment CYP2B1 was induced in foetal rats. The more recent investigations by Hulla and Juchan [44] suggest CYP3A1 to be inducible in foetal rat liver as early as day 15 of gestation in response to maternal treatment with PCN.

In the present study, a weak CYP3A1 cross-reactive protein was found with microsomes isolated from control pregnant animals, but a very significant induction of this protein was seen in response to Aroclor 1254 treatment (see Fig. 1). There was a diminished expression of CYP3A1 at the higher treatment dose. The strong induction of CYP3A1 was paralleled by a significant increase in CYP3A1 mRNA (Fig. 2). Transplacental transfer of PCB isomers and congeners at a maternal treatment dose of 100 mg/kg body weight Aroclor 1254 resulted in an induction of foetal hepatic CYP3A1 (Fig. 1). However, at the higher dose the expression of foetal CYP3A1 was abolished. This suggests a bimodal response in the expression of CYP3A1 in maternal and foetal animals to an ascending Aroclor 1254 dose. The northern blot analysis shown in Fig. 2 accords with the western blot analysis of foetal CYP3A1. The results illustrate how versatile PCBs are in modulating the induction of CYP3A1.

Treatment of rats with a dose of 600 μmol/kg body weight Aroclor 1254 resulted in the specific induction of 12-hydroxylation of lauric acid as reported previously [45]. Furthermore, when maternal rats received a low dose of 5 mg/kg body weight of either 2,2',4,4',5,5'-hexachlorobiphenyl or 3,3',4,4'-tetrachlorobiphenyl lactational transfer was found which resulted in an increase in immunoreactive CYP4A protein measured in the neonate [46]. This suggests that the induction of CYP4A may not require the rigid molecular substitution pattern of PCBs as reported for the induction of other P450 isoenzymes such as CYP1A, CYP2A and CYP2B proteins (see discussion above and Parkinson *et al.* [8]. There is additional evidence to suggest a synergistic mode of action when both PCBs are given in combination, as further increases in immunoreactive protein could be measured [46]. A recent report suggests [47] a mechanism of induction that involves binding of hypolipidemic drugs to a steroid hormone receptor. In the present study, microsomal proteins from control maternal and foetal animals did not cross-react with polyclonal antibodies raised to rat CYP4A1 (Fig. 1). However, treatment of maternal rats with Aroclor 1254 resulted in the significant induction of CYP4A1 at both dose levels employed (Fig. 1). Although transplacental transfer of PCB isomers and congeners resulted at the lower dose in the induction of foetal CYP4A1, the higher dose of 250 mg/kg body weight virtually abolished this response.

Northern blot analysis (Fig. 2) shows a decline in the maternal CYP4A mRNA transcript in response to PCB treatment. The induction of CYP4A1 (Fig. 1) may indicate post-transcriptional regulation of CYP4A by stabilization of the protein against degradation. Similar results have been obtained for CYP2A1, CYP2C7 and CYP2E1 (see above).

Recently, Jenke *et al.* [35] published a detailed report on the expression of protooncogenes in rat liver by feeding a diet supplemented with a mixture of PCBs. Of the 10 protooncogenes investigated, c-Ha-ras, c-erbA, c-raf and c-yes were increased at the mRNA level in adult rat liver. Similar results were obtained for weaning rat liver mRNA extracts, but c-erbB was additionally expressed in comparable experiments. This points to developmental differences in the expression of protooncogenes in adult and adolescent rats.

In the present study, developmental differences in the expression of protooncogenes were seen. Adult rats responded to PCB treatment with an increased c-raf expression, but foetal rats did not. In contrast, an increase in c-Ha-ras was seen in foetal rats, but maternal rats did not respond to Aroclor 1254 treatment. Unlike Jenke *et al.* [35], we were unable to detect erbA and erbB mRNA in liver extracts of the mother and the foetus. This points to physiological and developmental factors that might influence the expression and responsiveness of certain cellular protooncogenes to modulation by PCBs.

The induction of P450 proteins enhances the metabolism of PCBs and by implication reduces the cellular burden with these noxious chemicals. However, an increased metabolism of PCBs has been reported to be deleterious [48]. In particular,

binding of certain hydroxy-PCBs to the oestrogen receptor was reported to be detrimental. In contrast, Yoshimura *et al.* [49] have shown that metabolism of the toxic 3,3′,4,4′-tetrachlorobiphenyl was beneficial, as treatment of rats with the hydroxy-derivative produced a lower PCB toxicity, as judged on a number of biochemical and morphological indicators. Unfortunately, there are only a limited number of studies which compare the toxic responses of an animal to the parent PCB molecule and its metabolite. It is evident that the induction of CYP1A1 in animals may be considered as being disadvantageous, as its role in the metabolic activation of procarcinogens such as benzo[*a*]pyrene is unequivocal. Other factors such as displacement of physiological ligands from receptors by PCBs and their metabolites are likely to alter significantly the normal physiological functioning of cells, but presently, little is known about PCB receptor interaction. For instance, certain PCBs bind with high affinity to the Ah receptor, yet the physiological ligand for this receptor has not been identifed. Displacement of the natural ligand from this receptor is considered to produce a cascade of events leading to toxicity and perhaps cell death, but again little is known about the mechanism.

There is compelling evidence [50] that c-Ha-ras is implicated in the development of neoplasms of the colon, lung and pancreas. Furthermore, the protooncogenes erbB1 and erbB2 are implicated in squamous cell carcinoma, astrocytoma and adenocarcinoma of the breast, ovary and stomach. There is overwhelming evidence [51] that at an appropriate dose and for extended periods of time PCBs induce the formation of preneoplastic lesions, neoplastic nodules and hepatocellular carcinomas. In conclusion, the present study reports the concomitant expression of CYP isoenzymes and protooncogenes, but it is not known whether this expression represents a coordinated cellular mechanism of response. Further study is required to address this question.

Acknowledgement—The financial support of the Cancer Research Campaign of Great Britain to J. T. Borlakoglu is gratefully acknowledged.

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