Metabolic activation and DNA-adducts detection as biomarkers of chlorinated pesticide exposures

M. Dubois, Y. Grosse, J. P. Thomé, P. Kremers and A. Pfohl-Leszkowicz

Several authors have reported the high hepatic incidence of γhexachlorocyclohexane (γHCH), pentachlorophenol (PCP) and hexachlorobenzene (HCB) which are widely used as pesticides. Their genotoxicity status was not clearly known and no mutagenic effects, using the *Salmonella* assay, were reported. In the first part of this report, DNA-adduct formation is evaluated in three types of cultured hepatic cells (rodent, bird and human) as a biomarker of exposure to genotoxic compounds. γHCH–, PCP– and HCB–DNA adducts were analysed, using the sensitive ^{32}P-postlabelling assay in its nuclease P1 enrichment version. The genotoxicity of lindane and PCP is clearly established. Total DNA-adducts reached a maximum in foetal rat hepatocytes (17 and 15 adducts per 10^9 nucleotides) after an exposure to pentachlorophenol and lindane respectively. After HCB treatment, limited amounts of DNA-adducts were found in the different cells used. The finding that DNA adducts were not the same in all species tested might be due to metabolic differences. Each type of cultured cells preferentially express different cytochrome P450 families. These P450s metabolize a wide variety of xenobiotics and bioactivate carcinogens into reactive metabolites able to form DNA-adducts. The objective of the present study was to examine the possible association between DNA-adduction and particular CYP450 induction. The induced cytochrome P450s were measured by northern blot analysis. In rat and human cells, lindane treatment strongly induces CYP2B and CYP3A mRNA levels, whereas pentachlorophenol treatment induces CYP1A, CYP2B and CYP3A.

Keywords: *DNA adducts, ^{32}P-postlabelling, CYP450, pentachlorophenol, hexachlorocyclohexane, hexachlorobenzene.*

Introduction

Many xenobiotics may interact with DNA directly or after transformation by metabolizing enzymes into reactive electrophilic species able to covalently bind to nucleophilic sites in DNA, forming DNA addition products called adducts. The formation of a DNA-adduct is considered to be a critical step that may initiate carcinogenesis and mutagenesis (Miller and Miller 1981, Pfohl-Leszkowicz 1994).

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γHexachlorocyclohexane (γHCH or lindane), pentachlorophenol (PCP) and hexachlorobenzene (HCB) (Figure 1) are chlorinated compounds which are widely distributed as pesticides in the environment. They have a high lipophilicity, can accumulate in food chains and are found in all organisms of natural ecosystems as well as in human tissues (Engelhardt *et al.* 1986, Ferrando *et al.* 1992). They accumulate in the organism as long as they are not biotransformed by drug-metabolizing enzymes. The cytochrome P450 monooxygenases potentiate promutagens and procarcinogens by converting them into active genotoxic metabolites.

The biotransformations of these chlorinated chemicals are closely related. They have numerous toxic effects, the most prominent being hepatotoxicity, an alteration of porphyrin metabolism and induction of hepatic microsomal enzymes giving rise to reactive intermediates during biotransformation (De Matteis *et al.* 1961, Stonard and Nenov 1974, Aschmann *et al.* 1989, Hayes and Laws, 1991, Bainy *et al.* 1993, Colosio *et al.* 1993).

The genotoxicity status of PCP, γHCH and HCB was controversial since almost all microbial and mammalian assays were negative. Ashby and Tennant, in 1988, reported a 'no mutagenic effect' of lindane using a *Salmonella* assay, and negative results for carcinogenicity in rats and mice after an exposure via food. No increase in tumour incidence has been reported in rats, and lindane does not induce chromosomal breakage in mice and CHO (Chinese Hamster Ovary) cells (for review, see IPCS WHO 1991). Pentachlorophenol (50–100 mg kg^{-1}), appears also as non-mutagenic in the Ames test (Haworth *et al.* 1983). It does not induce damage on DNA in CHO cells (Ehrlich 1990) and Ziemsen *et al.*, in 1987, have shown that an occupational exposure of PCP did not induce sister chromatid exchange or chromosomal breakage.

In this study, we compare a culture of human hepatoma cells Hep G2, which mainly express *CYP3A7*, the major 3A family member present in the human foetal livers (Yang *et al.* 1994), with two types of foetal hepatocytes from rodents and avians. Foetal rat hepatocytes in primary culture were used as a reference model and foetal quail hepatocytes (*Coturnix coturnix japonica*) were used as an ecotoxicological 'witness'. It is a subspecies of quail (*Coturnix coturnix*), an omnivorous bird whose populations have considerably dwindled over the past decade due to the extensive use of pesticides in agriculture (Canters and Snoo 1995). Many studies have shown the inducibility of their hepatic monooxygenases (Safe 1984, Dalvi *et al.* 1987, Lubet *et al.* 1990, Leonzio *et al.* 1992).

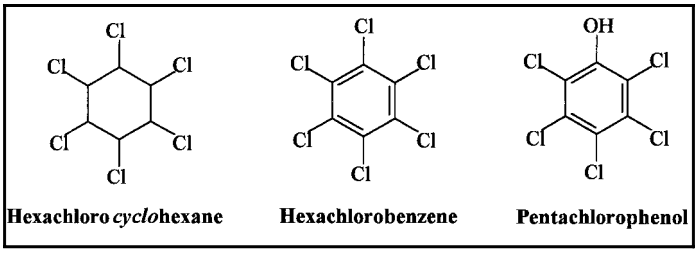

Figure 1. Structure of hexachlorocyclohexane, pentachlorophenol and hexachlorobenzene.

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In the first part of this report, DNA-adduct formation is investigated as a biomarker of exposure to possible genotoxic compounds. We searched and compared γHCH–, PCP– and HCB– DNA adducts formed in three species, using the sensitive ^{32}P-postlabelling assay (Randerath *et al.* 1981) in its nuclease P1 enrichment version. Most of the environmental chemicals are carcinogenic and mutagenic only after undergoing metabolic activation by a P450-linked monooxygenase system. The hepatic cytochrome P450s are mixed-function oxidases involved in the metabolism of a wide variety of xenobiotics and endobiotics, which unfortunately may also bioactivate carcinogens into reactive metabolites able to form DNA-adducts. Since the CYP450 are highly inducible, the second part of this report tries to establish a link between DNA-adduct formation and CYP1A, CYP2 and CYP3A induction by the above mentioned chlorinated chemicals.

MATERIALS AND METHODS

Chemicals

Proteinase K, ribonuclease A, T1 micrococcal nuclease and apyrase were obtained from Sigma (St Louis, MO, USA), spleen phosphodiesterase from Worthington Biochemicals (Freehold, NJ, USA), nuclease P1 from Boehringer (Mannheim, Germany), and T4 polynucleotide kinase and [γ^{32}P]-ATP, 5000 Ci mmole^{-1} (185 TBq mmole^{-1}), from Amersham (Buckinghamshire, UK). Cellulose MN 301 was from Machery-Nagel (Düren, Germany). PEI-cellulose plates were made in the laboratory. Culture media and newborn calf serum were from Gibco (Ghent, Belgium). Disposable materials used for cell cultures were obtained from Becton Dickinson (Helsinki, Finland). Pesticides were a Riedel De Haën product (Seelze, Germany) guaranteed 99% pure. All other chemicals (benzanthracene, phenobarbital) were analytical grade quality and purchased from Sigma (Munich, Germany).

Isolation and culture of hepatocytes

Foetal rat livers were collected on the 18th day of gestation and used to prepare primary cultures as described earlier (Kremers *et al.* 1990).

Quail eggs (*Coturnix coturnix japonica*) were obtained from a local breeding farm (Ferme du Moulin, Liernu, Belgium) and used to prepare the primary cultures after incubation for 16 days at 37 °C with a 12-h lighting cycle (Roelandt *et al.* 1995).

Hep G2 (human liver hepatoblastoma) cells were obtained from the American Type Culture Collection, HB 8065, Maryland. The culture medium was Dulbecco's Eagle Minimum Essential Medium (DMEM) containing sodium pyruvate, foetal calf serum 10% (v/v), and antibiotics (penicillin, streptomycin). Culture media were renewed every 24 h. Hep G2 cells were regularly subcultured after trypsinization and five-fold dilution. The monolayer cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2–95% air. Unless otherwise specified, foetal hepatocytes and Hep G2 cells were cultured in the presence of dexamethasone (10^{-6} M). As demonstrated earlier, this glucocorticoid maintains expression of the different cytochromes P450 and promotes the survival of the culture for several weeks (Bollinne *et al.* 1987).

Induction of drug-metabolizing enzyme activities in monolayers

Timing of cell treatment began at plating. Inducers were added to the culture media after 24 h and maintained in the cultures throughout the experiment (72 h). The stock solutions of benzanthracene (BA), dexamethasone (Dexa), phenobarbital (PB) and pesticides were prepared in dimethylsulphoxide (DMSO). As previously described, the final concentration of compounds in the culture medium was 50 μM (Dubois *et al.* 1996a). The final concentration of DMSO, used as a solvent in the medium, never exceeded 0.1% (v/v). A similar volume of solvent was added to control cultures.

^{32}P-postlabelling of DNA adducts

DNA (7 μg) was extracted and purified as described previously (Pfohl-Lezskowicz *et al.* 1993). The DNA concentration was measured spectrophotometrically in water solution using a value of 1 A_{260} units = 50 μg of DNA. The method used for ^{32}P-postlabelling was that previously described by Randerath *et al.* (1981), modified by Reddy and Randerath (1986). Briefly, digested DNA is treated with nuclease P1 before ^{32}P-postlabelling. Nuclease P1 dephosphorylates 3′-monophosphate of normal nucleotides, whereas adducts protect this binding. Only adduct-bearing 3′P-nucleotides are labelled by T4 polynucleotide kinase. Normal nucleosides and pyrophosphate were removed by overnight chromatography on polyethyleneimine cellulose in 2.3 M NaH_2PO_4 pH 5.7 (D1). Origin areas containing labelled adduct-bearing nucleotides were cut and transferred to another PEI-cellulose plate in 4.25 M lithium formate and 7.5 M urea, pH 3.35 (D2). Two further migrations (D3 and D4) were performed in the same direction, perpendicular to D2. The solvent for D3 was 0.6 M NaH_2PO_4 and 5.9 M urea, pH 6; the solvent for D4 was 1.6 M NaH_2PO_4, pH 6. Autoradiography was carried out at –80 °C for 3 days exposure in the presence of an intensifying screen (Cronex). Spots were scraped off, and their radioactivity was counted by the Cerenkov technique.

Northern blot analysis

Total RNA was extracted from Hep G2 cells, rodent and avian hepatocytes by the guanidium thiocyanate/CsCl method of Chirgwin *et al.* (1979). The RNA (20 μg) was size-fractionated on electrophoresis in a denaturing agarose gel and transferred to a positively charged nylon hybridization transfer membrane. CYP450 mRNAs were hybridized with cDNA oligonucleotides probes labelled to a specific activity of 10^7 cpm per μg by random priming. Final washing conditions for the cDNAs were 2 × standard saline citrate (20 × standard saline citrate = 3 M sodium chloride, 0.3 M sodium citrate, pH 7.5), 0.1% sodium dodecyl sulphate, for 15 min at 65 °C. We used cDNA probes coding for human CYP1A, CYP2E and CYP3A4 and for rat CYP1A1, CYP2B1 and CYP3A1. The blots were also probed with human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA ^{32}P-labelled by random priming. RNA bands were visualized by autoradiography and quantified by scanning densitometry.

Results

DNA-adduct formation

DNA-adduct patterns of control foetal rat, quail hepatocytes and Hep G2 cells are shown in Figure 2. No adducts are found in control foetal rat hepatocytes and Hep G2 cells. Unexpectedly, two adducts, namely spots 1 and 2, were detected in control quail DNA. Quantitative estimates of these spots are respectively 5.7 and 1.8 adducts per 10^9 nucleotides. Therefore, these values were always subtracted from the corresponding spots in the pattern of quail cells after pesticide treatments. The origin of these adducts is unknown, but they might have arisen by 'natural' contamination either of eggs or of the parents via their food.

The patterns of the adducts in hepatic cells after an exposure to chlorinated chemicals are shown in Figure 3. Quantitative estimates of the different adducts are given in Table 1. Total levels of DNA-adducts are highest in rat hepatocytes independently of the substances and represent 17 and 15 adducts per 10^9 nucleotides for pentachlorophenol and

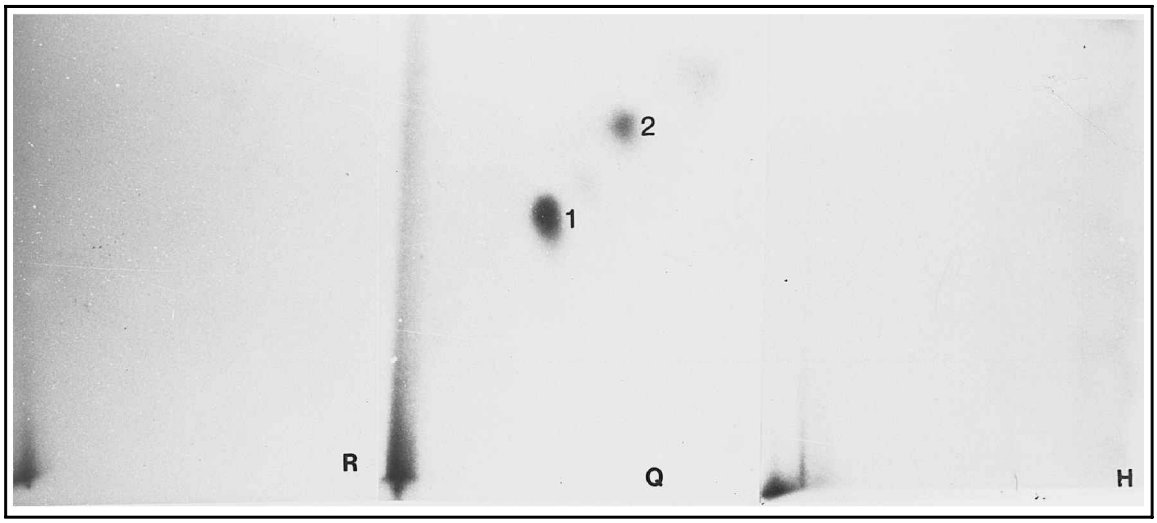

Figure 2. Autoradiograms of control DNA-adduct in control rat (R), quail (Q) and Hep G2 (H) cells.

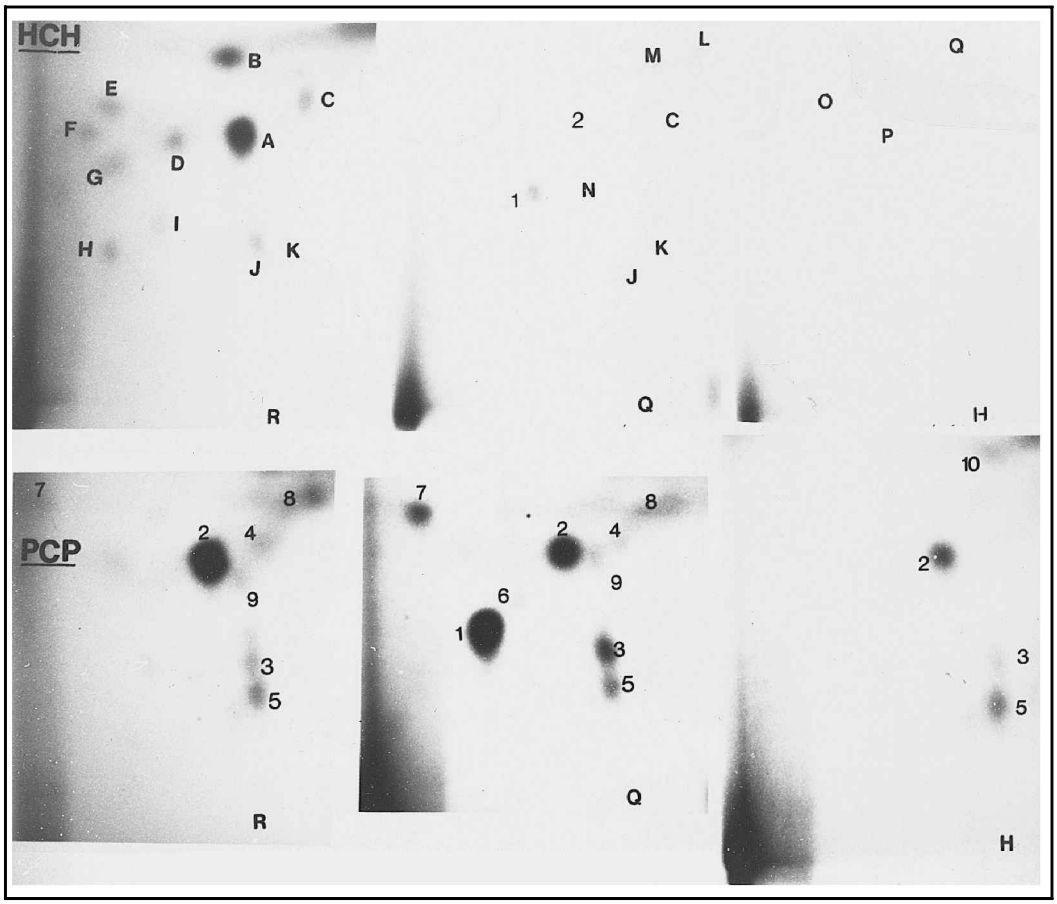

Figure 3. Autoradiograms of DNA-adducts, after exposure to a single dose (50 μM) of lindane (a) and pentachlorophenol (b) of rat (R), quail (Q) and Hep G2 (H) cells.

lindane respectively. The total DNA-adducts formation is two-fold lower in quail cells independently of the treatment and seven-fold and four-fold lower in Hep G2 cells after lindane and pentachlorophenol treatment respectively.

After lindane exposure (Figure 3(a)), in foetal rat hepatocytes there are more different DNA-adducts, 11 designated from A to K, than in foetal quail hepatocytes (nine adducts) and Hep G2 cells (three adducts). The major adducts are A in foetal rat hepatocytes and L in foetal quail cells. There are no major adducts in Hep G2 cells. Some adducts are specific to the type of cells (Hep G2 cells: O, P and Q; rat hepatocytes: A,B,D,E,F,G,H,I; and quail hepatocytes: L,M,N). Only three of them (C,J and K) are detected both in rat and quail hepatocytes.

Cells	Adducts of Pentachlorophenol (no. per 10^9 nucleotides)										
	1	2	3	4	5	6	7	8	9	10	Total
Rat	0	8.9	1	1.6	1.8	0	0.1	2.2	1.4	0	17
Quail	5.4	1.5	1.5	0.2	0.6	0.2	1	1.5	0	0	11.0
Hep G2	0	2	0.2	0	1	0	0	0	0	1	4.2

Cells	Adducts of Lindane (no. per 10^9 nucleotides)																
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	Total
Rat	3.6	2	1.9	1.1	0.8	1.3	1.1	0.6	1	1.2	0.1	0	0	0	0	0	0	14.8
Quail	0	0	1.8	0	0	0	0	0	0	0.3	1.3	2	1.2	0.8	0	0	0	7.4
Hep G2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0.7	0.8	2.2

Table 1. Quantification of pentachlorophenol– and lindane–DNA adducts in rat, quail and Hep G2 cells treated with a single dose of pesticide (50 μM). Results are expressed as number of adducts per 10^9 nucleotides.

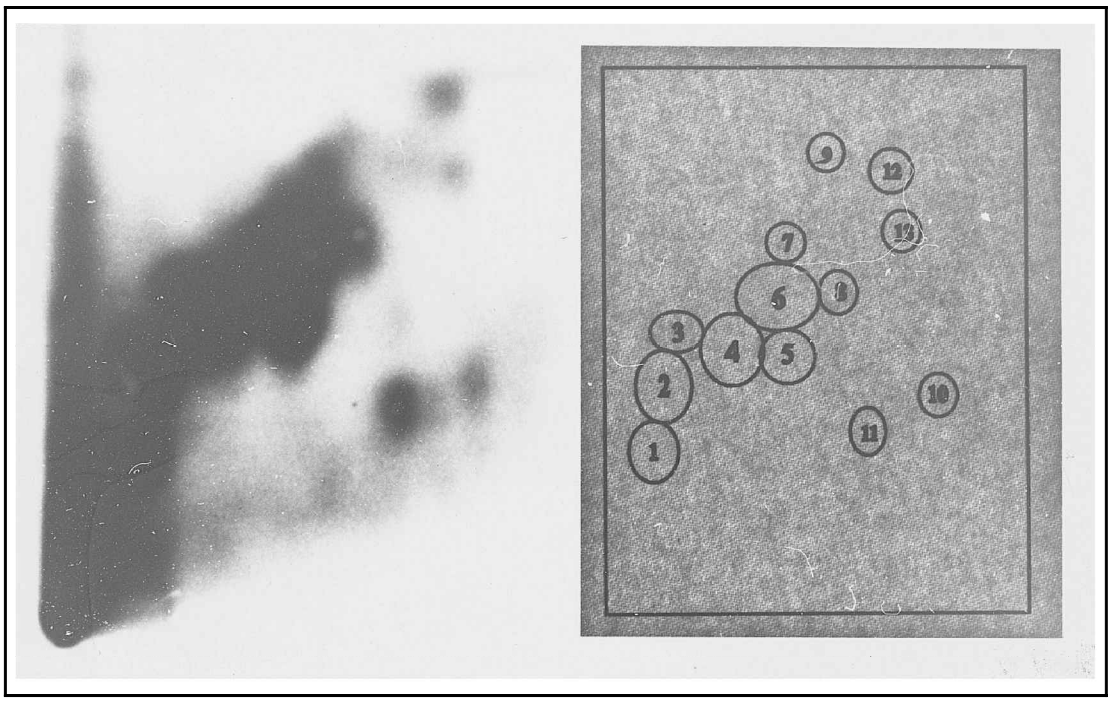

Figure 4. Autoradiograms of a positive reference of ^{32}P-postlabelled hepatic cellular DNA, digested from Hep G2 cells treated with a single dose of benzanthracene (50 μM).

After pentachlorophenol exposure, the pattern of DNA-adducts is very similar in the three types of cells (Figure 3(b)). Seven DNA-adducts are quantified in rat hepatocytes, nine in quail cells and four in Hep G2 cells. Some others could be observed but their levels are near the detection limit. Adduct no. 2 is dominant both in rat hepatocytes and Hep G2 cells. It represents 50% and 47% respectively of the total. A different major adduct (adduct no. 1) is found in foetal quail hepatocytes and represents 46% of the total. Some adducts (nos 2,3,5) are found in all types of cells, some appeared both in foetal rat and quail hepatocytes (nos 4,7,8). Some are specific to the cells (nos 1 and 6 in quail, no. 9 in rat hepatocytes, no. 10 in Hep G2 cells). After hexachlorobenzene treatment, the total number of DNA-adducts is very small and varies from 1.3 to 4.2 per 10^9 nucleotides in all cells (results not shown).

Benzanthracene (PAH; 50 μM), a known mutagen which is also metabolically activated by the cytochromes P450, is used as a positive control. With benzanthracene, the number of adducts was indeed very high, totalling 172–290 adducts per 10^9 nucleotides. One such pattern is shown in Figure 4. We found 12 spots common to all three types of hepatic cells after PAH treatment.

Analyses of cyt P450 mRNA

To determine which are the different CYP450 families induced by these three chlorinated pesticides, we used northern blot analysis to examine levels of the corresponding mRNAs in the three types of cultured cells. The cells were either left untreated (control), exposed to 50 μM of one of two known inducers, phenobarbital (PB) and benzanthracene (BA) (positive controls), or exposed to 50 μM of one of the

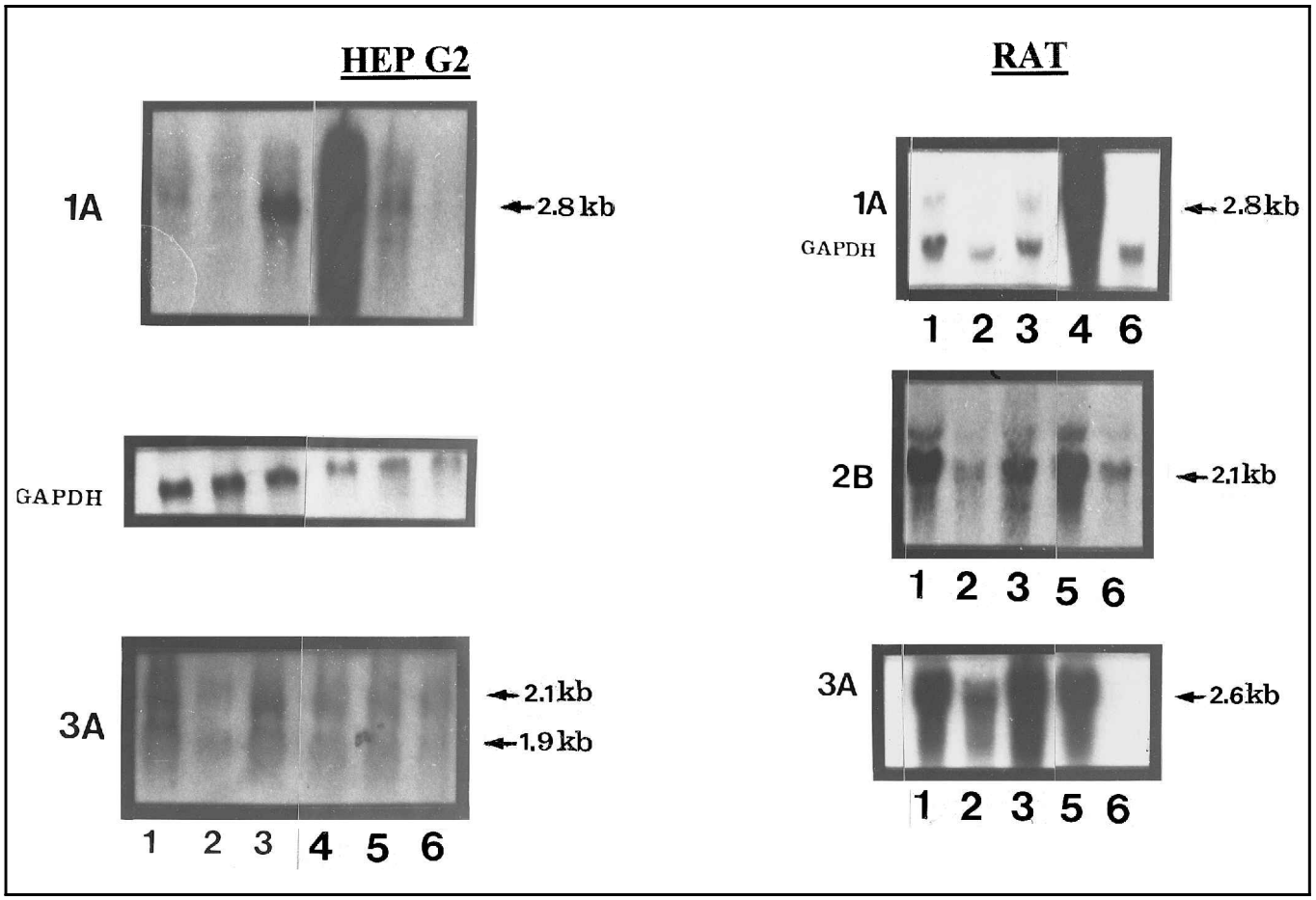

Figure 5. Northern blot analysis in cultured hepatic cells. Total cellular RNA (20 μg) was harvested from cultures of foetal hepatocytes and Hep G2 cells and analysed by northern blot hybridization as described under Materials and Methods. Molecular sizes were deduced from that of ethidium-bromide-stained 18S marker RNA (Bethesda Research Laboratory). Foetal rat hepatocytes and Hep G2 cells were treated with 50 μM (1) lindane, (2) hexachlorobenzene, (3) pentachlorophenol, (4) benzanthracene, or (5) phenobarbital. Lane (6) contains RNA from control cells. RNA was subjected to northern blot hybridization with rat *CYP1A1, CYP2B1* and *CYP3A1* and human *CYP1A, CYP2E* and *CYP3A4* cDNA probes. Total mRNA was also probed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The intensities of the mRNA in the autoradiograms were estimated by densitometry and normalized to GAPDH to confirm inducer effects.

pesticides. Figure 5 shows representative northern blots obtained with a specific rat CYP450 cDNA probe (on the right), a human specific CYP1A cDNA probe and a non-specific human CYP3A4 cDNA probe (+ 905 + 2036) able to recognize all the members of the CYP3A family (on the left). Results from control cells (lane 6), phenobarbital (lane 5) and benzanthracene (lane 4) induced cells are shown for comparison purposes. The small amount of dexamethasone (10^{-6} M) present in the culture medium is unable to induce CYP3A but allows the induction of the other P450s in cultured cells. Therefore, no hybridizable band of CYP3A mRNAs were found in control cells. On these blots, only pentachlorophenol exhibited a CYP1A specific signal (2.8 kb) in Hep G2 cells, while a weak hybridizable band (2.8 kb) was found in foetal rat hepatocytes after lindane and PCP treatments. Moreover, these treatments also led to intense hybridized bands of CYP2B1 (2.1 kb) and CYP3A1 (2.6 kb) in foetal rat hepatocytes and CYP3A (1.9 kb, 2.1 kb) in Hep G2 cells. An exposure to hexachlorobenzene only revealed an inductive effect on CYP3A1 transcript levels in the cultured foetal rat hepatocytes. In quail cells and under conditions of reduced stringency, we used a human CYP2E cDNA probe sharing the highest homology. In these conditions, a very weak hybridizable band was revealed only after a phenobarbital treatment (results not shown).

Discussion

To avoid some of the difficulties of experimentation in living animals, we selected three types of cells as possible systems to investigate the biotransformation of pesticides. Taken together, these cells express the main CYP450 families and provide a valuable panel for metabolic studies. We compared a human hepatoma cell line, Hep G2, which mainly expresses CYP3A7 and to a lesser extent CYP1A2 (Sassa *et al.* 1987, Roberts *et al.* 1990, Schuetz *et al.* 1993), with two types of foetal hepatocytes from rodent and avian species. Foetal quail hepatocytes are known to express *CYP2*-family genes (Lubet *et al.* 1990) displaying the greatest similarity to the *CYP2C* gene, constitutively expressed in rat liver (50–56% homology), and the *CYP2E* gene constitutively expressed in human liver (30–33% homology) (Hansen and May 1989, Sinclair *et al.*

1990, Lubet *et al.* 1990). A previous study has clearly demonstrated that, in our culture conditions, foetal rat hepatocytes express CYP1A1, CYP2B and CYP3A1 (Dubois *et al.* 1996a).

Exposure of hepatic cells to each of four different chemicals (i.e. BA, PCP, γHCH, HCB), results in a significant induction of liver CYP450 mRNA levels (specially CYP1A) paralleled by an increased formation of DNA-adducts. In rat cells, pentachlorophenol and lindane are revealed as mixed type inducers of CYP450 families. In Hep G2 cells, PCP induces higher CYP1A mRNA levels than lindane. These results confirm and complement those of a previous study conducted in our laboratory where, in treated-Hep G2 cells, we have clearly demonstrated the higher inductive effects of PCP on ethoxyresorufin-*O*-deethylase (EROD), a CYP1A1 specific enzymatic activity. In contrast, lindane mainly induced ethoxycoumarin-*O*-deethylase (ECOD) activity which is supported by different cytochrome P450s, namely CYP2B and CYP3A families (Dubois *et al.* 1996b). The significant inductive effect on CYP450 transcription levels of these chlorinated chemicals is closely related to the level of DNA-adduct formed: foetal rat hepatocytes are the most active in producing DNA-adducts after pentachlorophenol and lindane treatment and total DNA-adducts formed after pentachlorophenol treatment is always higher than after a lindane exposure in both hepatic cells.

In 1994, Moorthy *et al.* found high levels of DNA-adducts in benzanthracene- and benzo[*a*]pyrene-treated rat cells, indicating an increased conversion of these polycyclic aromatic hydrocarbons (PAH) to ultimate carcinogenic metabolites. So, the high similarity of the adduct pattern displayed by rat, quail and human cells after exposure to PCP supports the hypothesis that in these cells, this chemical is biotransformed by the same drug-metabolizing enzymes yielding the same genotoxic metabolites and thus the same DNA-adducts. Pentachlorophenol has no direct mutagenic effect using Ames *Salmonella* assays (Haworth *et al.* 1983). The main intermediates of pentachlorophenol biotransformation are *p*-tetrachlorohydroquinone (TCHQ) and 2,4,5-trichlorophenol (Renner and Hopfer 1990). Ehrlich, in 1990, has shown that TCHQ exerts a greater toxic effect on the growth of the CHO cells than PCP. It causes, at concentrations of 2–10 $\mu g\ l^{-1}$, DNA single strand breaks in intact cultured mammalian CHO cells, enhancing the toxicity of TCHQ to these cells. It can also be suggested that the formation of chromosomal aberrations in peripheral lymphocytes of workers exposed to PCP reported by Bauchinger *et al.* (1982), is caused by TCHQ after metabolism of PCP. Moreover 2,4,5-TCP has attained notoriety after the environmental catastrophe of Seveso in 1976, where the extremely toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was formed by condensation of two molecules of 2,4,5-TCP. In regard to this case, the observation of trace amounts of this dioxin among metabolites produced by animals treated with PCP was of significance (Renner and Mücke 1986). Therefore two compounds, TCHQ and TCDD, could be genotoxic and give the DNA-adducts pattern found in the three types of cell used.

In the same way, γhexachlorocyclohexane (lindane) is not directly mutagenic (Ashby and Tennant 1988). The patterns of DNA-adducts obtained in this study are very different in regard to the type of cells. Only three adducts of the total number are detected both in rat and quail hepatocytes and all the adducts formed in Hep G2 are specific. The finding that DNA-adducts are not quantitatively the same in all species tested might be due to differences in their metabolism, leading to different carcinogens, and to differences in the repair process (Pfohl-Lezskowicz *et al.* 1993). Biotransformation of lindane is initiated by dehydrogenating, dechlorinating, dehydrochlorinating and hydroxylating steps (Hayes and Laws 1991). Human liver microsomes converted lindane into four major metabolites γhexachlorocyclohexene (HCCH), γpentachlorocylcohexene (PCCH), βPCCH and 2,4,6-trichlorophenol (2,4,6-TCP). Smaller amounts of 2,3,4,6-tetrachlorophenol, pentachlorobenzene and pentachlorophenol were also found (IPCS WHO 1991). In 1990, De Marini *et al.* characterized the genotoxicity of these compounds, namely clastogenicity, their association with cancer and chromosomal aberrations in humans (PCP) and their carcinogenicity in rodents (2,4,6-TCP and PCP). In accordance with our results, a comparison between lindane and PCP patterns, from their migration characteristics, shows that the adducts A and 2 are similar in foetal rat hepatocytes and represent a common metabolite of lindane and pentachlorophenol. After PCP treatment, it represents 50% of the total DNA-adduct, whereas, adduct A after a lindane treatment represents only 24% of the total.

In this study, hexachlorobenzene appears as a non-genotoxic compound and as a preferential inducer of CYP3A1 mRNA in foetal rat hepatocytes. HCB does not induce CYP450 in Hep G2 cells. Many authors have studied the metabolism of lindane and pentachlorophenol and established similarities in their biotransformation pathways (for review, see Renner and Mücke 1986). However, even if in some cases PCP was found to be a metabolite of HCB in rat liver microsomes, this conversion never exceeds 1% and needs strong HCB-pretreatment of the animals *in vivo* (Van Ommen *et al.* 1985). The first step in the metabolic pathways of HCB is the reaction with glutathione and biotransformation into non-toxic conjugated intermediates (Renner and Hopfer 1990).

To conclude, it is noteworthy that these results are in agreement with a hierarchical classification (cluster analysis) previously made in regard to the induction of enzymatic activities obtained after pesticide exposures of the same hepatic cells (Dubois *et al.* 1996b). This study has shown that the strongest inducers of the enzymatic activities are pentachlorophenol and benz[*a*]anthracene (our positive reference in this report). Lindane has also a strong inductive effect and hexachlorobenzene appeared as an inert compound.

Taken together, the results highlight the strong hepatic incidence of lindane and pentachlorophenol through their biotransformation pathways and a high inductive effect of CYP1A, CYP2B and CYP3A families. Our study provides further evidence that combined investigation of DNA-adduct formation and cytochrome P450 induction in hepatic cells

constitutes a suitable means to investigate xenobiotic biotransformation and predict health risks in humans. Nevertheless, more investigations *in vivo* and *in vitro* are needed to unravel (1) the metabolic pathways leading to different adducts in different species, (2) the nature of the modified bases and the nature of the compounds bound to DNA, in order to better understand the mechanism of genotoxicity and carcinogenicity of pesticides.

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