Chem.-Biol. Interactions, 9 (1974) 379–387

© Elsevier Scientific Publishing Company, Amsterdam—Printed in The Netherlands

ENHANCEMENT OF MICROSOMAL DRUG OXIDATION AND GLUCURONIDATION IN RAT LIVER BY AN ENVIRONMENTAL CHEMICAL, POLYCHLORINATED BIPHENYL

HARRI VAINIO

Department of Physiology, University of Turku, Turku 52 (Finland)

(Received April 15th, 1974)
(Accepted July 25th, 1974)

SUMMARY

A polychlorinated biphenyl (PCB) compound, Clophen A 50, enhanced both hepatic aryl hydrocarbon hydroxylase and *p*-nitroanisole *O*-demethylase activities (*7.5-fold and 16-fold, respectively*), after treating the rats for 6 days with consecutive daily injections of Clophen A 50 (15 mg/kg i.p.). The treatment increased 3-fold the content of the carbon monoxide binding hemoprotein in liver microsomes, causing a concomitant shift in its reduced carbon monoxide absorbance peak to 448 nm. NADPH cytochrome *c* reductase, another component reaction of the microsomal mixed-function oxidase, was enhanced 1.5-fold in 6 days. A slight enhancement in the overall hydroxylation reactions was already observable 24 h after a single injection of Clophen A 50.

The UDPglucuronosyltransferase activity of native liver microsomes was enhanced 3-fold in 6 days by the Clophen A 50 treatment of rats. The enhancement was, however, more pronounced, if the microsomes were treated *in vitro* with membrane-perturbing agents to activate the latent UDPglucuronosyltransferase before measuring its activity. After treatment for 6 days, the enhancement was about 6-fold in digitonin-treated, 5-fold in phospholipase C-treated and about 10-fold in trypsin-digested microsomes. No enhancement could be detected 24 h after a single Clophen A 50 injection.

Aryl hydrocarbon hydroxylase activity was also enhanced in lung (5-fold), and kidney (8-fold) microsomes, whereas the microsomes from the duodenal mucosa exhibited no enhancement by a Clophen A 50 treatment of rats for 3 days.

The data obtained support the assumption that PCBs form a new type of inducer group in enhancing the microsomal drug biotransformation. Both the monooxygenase complex and UDPglucuronosyltransferase differ in their properties from those after

Abbreviation: PCB, *polychlorinated biphenyl.*

MONS 081833

enhancement with the known types of inducers, exemplified by phenobarbital and 3-methylcholanthrene, respectively.

INTRODUCTION

PCBs are widely used industrial chemicals, residues of which have been found in tissues of many species of birds and fish[1-4]. Contamination of human adipose tissue and human milk have also been detected[5,6]. Although PCBs have been used for more than 30 years, detailed studies of their toxicological properties have only recently been undertaken.

It has been suggested that PCBs represent a new type of inducer of drug monooxygenase system, differing in their properties from the two documented groups (of which phenobarbital and 3-methylcholanthrene are prototypes)[7]. Both phenobarbital and 3-methylcholanthrene are known to enhance drug oxidation and glucuronidation in rat liver[8-12]. However, no studies have been published on the effect of polychlorinated biphenyls on the glucuronidation step of drug metabolism. The present study examines the properties of a PCB compound as an inducer of the microsomal biotransformation reactions (mixed-function oxidase and its component reactions, and UDP-glucuronosyltransferase). Advantage was also taken of the *in vitro* treatment of microsomes with a surfactant digitonin, phospholipase C, and trypsin in order to reveal the possible latent UDPglucuronosyltransferase after treating the rats with polychlorinated biphenyls[11-14].

MATERIALS AND METHODS

Male Wistar rats (total number 44, weight about 200 g), fed *ad libitum*, were used. The PCB mixture used was Clophen A 50 (50% chlorinated, Oy Suomen Bayer, Helsinki, obtained as gift from the State College of Veterinary Medicine). Clophen A 50 (15 mg/kg as a daily dose), dissolved in corn oil, was administered intraperitoneally for 1 to 6 days. Control rats received corn oil only (5 ml/kg per day). The rats were killed 24 h after the last injection of Clophen A 50 by a blow on the head. The renal vessels were cut, the organs concerned were removed and microsomes prepared by the $CaCl_2$-aggregation method as earlier described[12]. The duodenal mucosal cells were scraped from the gut wall before homogenization. The digitonin treatment of liver microsomes was done essentially as indicated by VAINIO AND ATTIO[12]. The trypsin (twice crystallized, type XII from Sigma Chemical Co., St. Louis, Mo., U.S.A.) digestion of hepatic microsomes was done as previously described[11,13]. Phospholipase C digestion (150 μg, type I from *Cl.welchii;* Sigma Chemical Co.) of microsomes was conducted according to VAINIO AND HÄNNINEN[14]. Protein contents were determined by the biuret method[15].

The aryl hydrocarbon hydroxylase (EC 1.14.14.2) activity in hepatic microsomes was determined with the method of NEBERT AND GELBOIN[16] using 3,4-benzpyrene (0.16 m*M* in acetone) as substrate[11]. The incubation time varied between 5

MONS 081834

TABLE I

EFFECT OF PCB (CLOPHEN A 50) ADMINISTRATION (15 mg/kg i.p. DAILY) ON HEPATIC MICROSOMAL ENZYMES, ON PROPORTIONAL LIVER WEIGHT AND ON MICROSOMAL PROTEIN CONTENT PER g OF LIVER IN RAT

The concentrations of cytochrome P-450 (P-448) and the activities of NADPH cytochrome *c* reductase (activity expressed as nmole cytochrome *c* reduced/min × g protein), *p*-nitroanisole *O*-demethylase (activity expressed as nmole *p*-nitrophenol formed/h × mg protein) and benzpyrene hydroxylase (activity expressed as nmole hydroxylated benzpyrene formed/h × mg protein) are given. The means ± standard errors of 4–6 rats are indicated.

Administration (doses)	*Liver/body weight (%)*		*Microsomal protein (mg/g liver wt)*		*NADPH Cyt.c reductase*		*Cyt. P-450 (448) (nmole/mg protein)*		*p-Nitroanisole O-demethylase*		*Benzpyrene hydroxylase*	
	Oil	*PCB*	*Oil*	*PCB*	*Oil*	*PCB*	*Oil*	*PCB*	*Oil*	*PCB*	*Oil*	*PCB*
1 (24 h)	4.0 ± 0.1	4.2 ± 0.1	19.4 ± 0.8	22.0 ± 0.8	94 ± 11	100 ± 8	0.30 ± 0.02	0.38 ± 0.04	18 ± 1	35 ± 5[a]	2.5 ± 0.3	4.3 ± 0.5[a]
3	4.1 ± 0.1	4.8 ± 0.1[a]	19.0 ± 1.0	22.5 ± 0.5[a]	93 ± 9	129 ± 8[a]	0.35 ± 0.02	0.62 ± 0.03[c]	23 ± 3	125 ± 12[c]	3.8 ± 0.7	16.5 ± 1.4[c]
6	4.0 ± 0.1	5.0 ± 1.0[c]	20.1 ± 1.0	24.6 ± 0.3[b]	96 ± 9	148 ± 8[b]	0.34 ± 0.02	0.96 ± 0.10[c]	18 ± 1	285 ± 11[c]	3.5 ± 0.4	26.3 ± 0.6[c]

[a] $2P < 0.05$.
[b] $2P < 0.01$.
[c] $2P < 0.001$.

MONS 081835

and 20 min depending on the tissue concerned. The *p*-nitroanisole *O*-demethylase activity was measured as described by VAINIO[11]. NADPH cytochrome *c* reductase was determined as earlier described[17,18]. Cytochrome P-450 content was determined by the method of OMURA AND SATO[19] from the carbon monoxide difference spectrum of dithionite reduced microsomes, using an absorbance coefficient of 91 mM^{-1} cm^{-1} between 450 or 448 nm and 490 nm.

The UDPglucuronosyltransferase (EC 2.4.1.17) activity with *p*-nitrophenol (0.5 m*M*) as aglycone was measured as described earlier using a 2.2 m*M* uridine diphosphoglucuronic acid (UDPGlcUA, obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.) concentration, in the presence of 10 m*M* potassium-EDTA[20].

The statistical significances have been computed with Student's *t*-test.

RESULTS

Treatment of rats with PCBs caused a significant increase in the liver/body weight ratio (Table I). At the same time, the content of Ca^{2+}-sedimented microsomes per g of fresh liver weight was also significantly increased.

The overall hydroxylation reactions, aryl hydrocarbon hydroxylase and *p*-nitroanisole *O*-demethylase were both enhanced in the liver by administration of Clophen A 50 to rats. In 6 days, the enhancements were 7.5-fold and 16-fold, respectively. Even 24 h after the injection of Clophen A 50 the overall hydroxylation reactions were significantly over the control level (Table I).

Of the component reactions of the microsomal mixed-function oxidase, especially the content of the carbon monoxide binding cytochrome was increased by Clophen A 50. The hemoprotein was observed to have its absorption maximum in its reduced carbon monoxide spectrum at 448 nm. The other component in mixed function oxidase system, NADPH cytochrome *c* reductase, was enhanced only 1.5-fold in 6 days of PCB treatment (Table I).

Aryl hydrocarbon hydroxylase activity was measured also from other organs, *i.e.* in microsomes isolated from lung, kidney and duodenal mucosa. PCBs were able after three consecutive daily injections to enhance the aryl hydrocarbon hydroxylase

TABLE II

THE EFFECT OF PCB MIXTURE, CLOPHEN A 50 ON THE EXTRAHEPATIC ARYL HYDROCARBON HYDROXYLASE ACTIVITY

Clophen A 50 was administered i.p. once daily (15 mg/kg) for three days. The means and standard errors of 4 experiments are indicated.

Organ	*Aryl hydrocarbon hydroxylase (nmole hydroxylated benzpyrene formed/h/mg protein)*	
	Control	*PCB-treated*
Kidney	0.056 ± 0.005	0.431 ± 0.062*
Lung	0.050 ± 0.014	0.256 ± 0.021*
Duodenal mucosa	0.089 ± 0.021	0.088 ± 0.031

*$P < 0.001$.

MONS 081836

TABLE III

EFFECT OF PCB (CLOPHEN A 50) ADMINISTRATION (15 mg/kg i.p. DAILY) ON HEPATIC MICROSOMAL UDPGLUCURONOSYLTRANSFERASE ACTIVITY AS MEASURED FROM NON-TREATED, DIGITONIN-, PHOSPHOLIPASE C- OR TRYPSIN-TREATED PREPARATIONS

The results are given as nmole *p*-nitrophenol bound/h/mg microsomal protein. Means and standard errors are indicated. Number of rats in brackets.

Administration (doses)	*Non-treated microsomes*		*Digitonin-treated*		*Phospholipase C-treated*		*Trypsin-treated*	
	Oil	*PCB*	*Oil*	*PCB*	*Oil*	*PCB*	*Oil*	*PCB*
1 (4) (24 h)	17 ± 3	20 ± 5	240 ± 16	253 ± 15	68 ± 7	78 ± 16	88 ± 5	128 ± 18
3 (6)	16 ± 1	27 ± 3	217 ± 13	774 ± 89[b]	74 ± 9	195 ± 27[a]	101 ± 13	429 ± 29
(6)	16 ± 2	39 ± 2[b]	233 ± 14	1328 ± 149[b]	93 ± 17	493 ± 31[b]	95 ± 18	957 ± 120[b]

[a] $P < 0.01$.
[b] $P < 0.001$.

MONS 081837

activity in lung and kidney microsomes (5-fold and 8-fold, respectively), whereas no significant change could be detected in the duodenal mucosa (Table II).

UDPGlucuronosyltransferase activity in liver microsomes was increased about 3-fold in 6 days when measured from the "native" microsomes (Table III). If the microsomes were treated *in vitro* before determination of the transferase activity with membrane-perturbing agents such as surfactant digitonin, trypsin, or phospholipase C, a much more pronounced enhancement in the specific activity could be detected. In trypsin-predigested vesicles the increase in measurable enzyme activity was about 10-fold after treatment for 6 days, in digitonin pretreated vesicles about 6-fold, and in phospholipase C-treated vesicles about 5-fold. No significant enhancement could be detected 24 h after a single injection of Clophen A 50.

DISCUSSION

The ability of polychlorinated biphenyls to enhance liver microsomal enzymes was first suggested by RISEBROUGH *et al.*[1] and STREET *et al.*[21]. ALVARES *et al.*[7] have suggested that PCBs would be a new type of inducer group of mixed-function oxidase, *i.e.* their inducing properties differed from the two known types of inducers in enhancing benzpyrene hydroxylase and ethylmorphine *N*-demethylase. One group of inducers, of which phenobarbital is a prototype, enhances the metabolism of a large variety of substrates by liver cells, a second group stimulates the metabolism of only a few substrates. Polycyclic hydrocarbons, such as 3,4-benzpyrene and 3-methylcholanthrene, comprise the second group of inducers. In addition, the effects of these compounds on cytochrome P-450 differ. Treatment of rats with phenobarbital increases the concentration of the P-450 hemoprotein in liver microsomes. However, 3-methylcholanthrene induces the formation of cytochrome P-448, a hemoprotein that differs in its spectral properties and catalytic activities from cytochrome P-450 present in untreated or in rats treated with phenobarbital[22–24]. PCBs were observed to produce a cytochrome P-448 with catalytic properties differing from those of P-448 hemoprotein induced by polycyclic hydrocarbons. The present data support the above assumption in showing that the P-448 hemoprotein as formed in Clophen A 50-treated rats increased the demethylation of *p*-nitroanisole more than the oxidation of 3,4-benzpyrene. PCBs were also able to increase the NADPH cytochrome *c* reductase activity which is known to be uninfluenced by polycyclic hydrocarbons. The PCB mixture used increased both the liver/body-weight ratio and the content of microsomes per g of liver (*cf.* refs. 25,26), similarly to phenobarbital group of inducers. Clophen A 50 is a mixture of PCBs comprising compounds of both high and low chlorine content. Since Clophen A 50 shows the inducing properties of both the barbiturate and polycyclic hydrocarbon classes of inducers, it is possible that Clophen A 50 contains chemicals having properties out of both classes of inducers.

Clophen A 50 was observed to be active in enhancing the aryl hydrocarbon hydroxylase in many organs studied, *i.e.* in liver, kidney and lung. Only in the intestinal mucosa was the activity of aryl hydrocarbon hydroxylase not enhanced by the PCB treatment. This may be due to inactivation of the aryl hydrocarbon hydroxylase

MONS 081838

enzyme complex by the proteases present in the mucosal layer[27]. It may also be due to a true tissue specificity in PCB induction. Earlier it has been found that many compounds, *e.g.* phenobarbital and chlorpromazine, have a restricted tissue specificity in their induction[20,28,29].

Barbiturates and polycyclic hydrocarbons both stimulate the UDPglucuronosyltransferase activity of liver microsomes probably by different mechanisms. The barbiturate-induced enhancement is best revealed by treating the microsomal membranes by proteolytic enzymes, *e.g.* trypsin[11]. The polycyclic hydrocarbon-enhanced UDPglucuronosyltransferase can be revealed by many agents, especially with membrane perturbants acting on the lipid phase of the membrane[12,14].

The Clophen A 50-induced increase in UDPglucuronosyltransferase activity could be detected by all of the perturbants studied, *e.g.* digitonin, trypsin and phospholipase C. The biggest difference between corn oil-treated and Clophen A 50-treated rats was obtained in the trypsin-treated microsomes, *i.e.* 10-fold. The treatment with PCBs, as well as *e.g.* phenobarbital, increases the latency of the membrane-bound UDPglucuronosyltransferase. The location of this enzyme in the microsomal vesicle membrane has been suggested to be behind a permeability barrier[30]. The enhancement in UDPglucuronosyltransferase activity as caused by Clophen A 50 was thus considerably greater than obtained by any of the previous inducers, both when measured in non-activated and in activated microsomes (*cf.* refs. 10, 11, 13).

The aryl hydrocarbon hydroxylase has been extensively studied lately because of its relationship to the field of oncology. Polycyclic hydrocarbons have been shown to be potent inducers of aryl hydrocarbon hydroxylase both in experimental animals[31,32] and in man[33]. It is nowadays believed that a metabolic activation of chemical carcinogens is required to form a chemically reactive, electrophilic ultimate carcinogen[34]. It has been established that arene oxides (epoxides) are the activated metabolites of certain polycyclic hydrocarbons[35–37]. The formation of epoxides is usually carried out by the cytochrome P-450 (448) cored mixed-function oxidase system. PCBs possessing some properties of certain polycyclic hydrocarbons, may be of considerable importance in increasing the cancer risk of the environment[26,38].

ACKNOWLEDGEMENTS

The able technical assistance of Mrs. RAIJA SÖDERHOLM and Miss LEENA RUOKONEN is gratefully acknowledged. The work was supported by Grants from the U.S. Public Health Service (AM-06018-12) and from the Finnish Cancer Foundation.

REFERENCES

1 R. W. RISEBROUGH, P. RIECHE, D. P. PEAKALL, S. G. HERMAN AND M. N. KIRVEN, Polychlorinated biphenyls in the global ecosystem, *Nature*, 220 (1968) 1098–1102.
2 S. JENSEN, A. G. JOHNELS, M. OLSSON AND G. OTTERLIND, DDT and PCB in marine animals from Swedish waters, *Nature*, 224 (1969) 247–250.
3 J. H. KOEMAN, M. C. T. N. DE BRAUW AND R. H. DE VOS, Chlorinated biphenyls in fish, mussels and birds from the river Rhine and the Netherlands coastal area, *Nature*, 221 (1969) 1126.

MONS 081839

4 C. A. Bache, J. W. Sirum, W. D. Younis and D. J. Lisk, Polychlorinated biphenyl residues: Accumulation in Cayuga lake trout with age, *Science*, 177 (1972) 1191.
5 L. Acker and E. Schulte, Über das Vorkommen von chlorierten Biphenylen und Hexachlorbenzol neben chlorierten Insektiziden in Humanmilch und menschlichem Fettgewebe, *Naturwissenschaften*, 57 (1970) 497.
6 F. J. Biros, A. C. Walker and A. Medbery, Polychlorinated biphenyls in human adipose tissue, *Bull. Environ. Contam. Toxicol.*, 5 (1970) 317–323.
7 A. P. Alvares, D. R. Bickers and A. Kappas, Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver, *Proc. Natl. Acad. Sci. (U.S.)*, 70 (1973) 1321–1325.
8 A. H. Conney, Pharmacological implications of microsomal enzyme induction, *Pharmacol. Rev.* 19 (1967) 317–366.
9 H. Remmer, Induction of drug metabolizing enzymes in the liver, *European J. Clin. Pharmacol.*, 5 (1972) 116–136.
10 A. Aitio, H. Vainio and O. Hänninen, Enhancement of drug oxidation and conjugation by carcinogens in different rat tissues, *FEBS Letters*, 24 (1972) 237–240.
11 H. Vainio, Drug hydroxylation and glucuronidation in liver microsomes of phenobarbital-treated rats, *Xenobiotica*, 3 (1973) 715–725.
12 H. Vainio and A. Aitio, Enhancement of microsomal drug hydroxylation and glucuronidation in rat liver by phenobarbital and 3-methylcholanthrene in combination, *Acta Pharmacol. Toxicol.*, 34 (1974) 130–140.
13 H. Vainio, Enhancement of hepatic microsomal drug oxidation and glucuronidation in rat by 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT), *Chem.-Biol. Interactions*, 9 (1974) 7–14.
14 H. Vainio and O. Hänninen, A comparative study on drug hydroxylation and glucuronidation in liver microsomes of phenobarbital and 3-methylcholanthrene-treated rats, *Acta Pharmacol. Toxicol.*, 35 (1974) 65–75.
15 E. Layne, Spectrophotometric and turbidimetric methods for measuring proteins, in S. P. Colowick and N. O. Kaplan (Eds.), *Methods in Enzymology*, Vol. 3, Academic Press, New York, 1957, pp. 447–454.
16 D. W. Nebert and H. V. Gelboin, Substrate inducible microsomal aryl hydroxylase, II. Cellular responses during enzyme induction, *J. Biol. Chem.*, 243 (1968) 6250–6261.
17 A. H. Phillips and R. G. Langdon, Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: Isolation, characterization, and kinetic studies, *J. Biol. Chem.*, 237 (1962) 2652–2660.
18 H. Vainio and O. Hänninen, Effect of surfactants on drug metabolism in hepatic microsomes, *Suomen Kemistilehti*, B 45 (1972) 56–61.
19 T. Omura and R. Sato, The carbon-monoxide binding pigment of liver microsomes, II. Solubilization, purification, and properties, *J. Biol. Chem.*, 239 (1964) 2379–2385.
20 O. Hänninen, On the metabolic regulation in the glucuronic acid pathway in rat tissues, *Ann. Acad. Sci. Fenn.*, Ser. A II, No. 142 (1968) 1–96.
21 J. C. Street, F. M. Urry, D. J. Wagstaff and A. D. Blau, Comparative effects of polychlorinated biphenyls and organochlorine pesticides in induction of hepatic microsomal enzymes, *Am. Chem. Soc. 158th Meet., Sept. 1969, Div. Pesticide Chem.* (Abstr. 017).
22 N. E. Sladek and F. J. Mannering, Evidence for a new P-450 hemoprotein in hepatic microsomes from methylcholanthrene treated rats, *Biochem. Biophys. Res. Commun.*, 24 (1966) 668–674.
23 A. P. Alvares, G. Schilling, W. Levin and R. Kuntzman, Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene, *Biochem. Biophys. Res. Commun.*, 29 (1967) 521–526.
24 A. Y. H. Lu, R. Kuntzman, S. West, M. Jacobson and A. H. Conney, Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds, and endogenous substrates, II. Role of cytochrome P-450 and P-448 fractions in drug and steroid hydroxylations, *J. Biol. Chem.*, 247 (1972) 1727–1734.
25 J. R. Allen and D. H. Norback, Polychlorinated biphenyl- and triphenyl-induced gastric mucosal hyperplasia in primates, *Science*, 179 (1973) 498–499.
26 C. L. Litterst, T. M. Farber, A. M. Baker and E. J. van Loon, Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat, *Toxicol. Appl. Pharmacol.*, 23 (1972) 112–122.
27 Ch. Lehmann, V. Ullrich and W. Rummel, Phenobarbital inducible drug monooxygenase activity in the small intestine of mice, *Arch. Pharmacol.*, 276 (1973) 89–98.
28 A. Aitio, Induction of UDP glucuronyltransferase in the liver and extrahepatic organs of the rat, *Life Sci.*, 13 (1973) 1705–1713.

MONS 081840

29 H. Vainio and E. Hiltanen, Drug metabolism in Gunn rats: Inability to increase bilirubin glucuronidation by phenobarbital pretreatment, *Biochem. Pharmacol.*, (1974) in press.
30 H. Vainio, *On the Topology and Synthesis of Drug-metabolizing Enzymes in Hepatic Endoplasmic Reticulum*, Dissertation, Turku, 1973, pp. 1–46.
31 A. H. Conney, E. C. Miller and J. A. Miller, Substrate-induced synthesis and other properties of benzpyrene hydroxylase in rat liver, *J. Biol. Chem.*, 228 (1957) 753.
32 L. W. Wattenberg, J. L. Leong and P. J. Strand, Benzpyrene hydroxylase activity in the gastrointestinal tract, *Cancer Res.*, 22 (1962) 1120–1125.
33 A. P. Alvares, A. Kappas, W. Levin and A. H. Conney, Inducibility of benzo(*a*)pyrene hydroxylase in human skin by polycyclic hydrocarbons, *Clin. Pharmacol. Therap.*, 14 (1973) 30–40.
34 J. A. Miller, Carcinogenesis by chemicals: An overview; G. H. A. Clowes Memorial Lecture, *Cancer Res.*, 30 (1970) 559–576.
35 H. Marquardt, T. Kuroki, E. Huberman, J. K. Selkirk, C. Heidelberger, P. L. Grover and P. Sims, Malignant transformation of cells derived from mouse prostate by epoxides and other derivatives of polycyclic hydrocarbons, *Cancer Res.*, 32 (1972) 716–720.
36 H. Marquardt and C. Heidelberger, Influence of "feeder cells" and inducers and inhibitors of microsomal mixed-function oxidases on hydrocarbon-induced malignant transformation of cells derived from C3H mouse prostate, *Cancer Res.*, 32 (1972) 721–725.
37 N. Kinoshita and H. V. Gelboin, The role of aryl hydrocarbon hydroxylase in 7,12-dimethylbenz(*a*)anthracene skin tumorigenesis: On the mechanism of 7,8-benzoflavone inhibition of tumorigenesis, *Cancer Res.*, 32 (1972) 1329–1339.
38 H. Nagasaki, S. Tomii, T. Mega, M. Marugami and N. Ito, Hepatocarcinogenicity of polychlorinated biphenyls in mice, *Gann*, 63 (1972) 805.

MONS 081841