Biochemical Pharmacology, Vol. 31, No. 15, pp. 2523–2529, 1982.
Printed in Great Britain.

0006-2952/82/152523-07 $03.00/0

INDUCTION OF LIVER MICROSOMAL CYTOCHROME P-450 AND ASSOCIATED MONOOXYGENASES BY OCTACHLOROSTYRENE IN INBRED STRAINS OF MICE

LACK OF CORRELATION WITH THE MURINE *Ah* LOCUS

JØRN A. HOLME and ERIK DYBING
Department of Toxicology, National Institute of Public Health, Postuttak, Oslo 1, Norway

(*Received* 19 *November* 1981; *accepted* 3 *March* 1982)

Abstract—Single intraperitoneal injections of octachlorostyrene (OCS) and hexachlorobenzene in genetically polycyclic aromatic hydrocarbon 'responsive' C57/BL/6 (B6) mice led to a time- and dose-dependent increase in the levels of liver microsomal cytochromes P-450 and b_5 as well as in the activities of NADPH cytochrome P-450 (cytochrome *c*) reductase, ethylmorphine (EM) *N*-demethylase, 4-nitroanisole (PNA) *O*-demethylase and acetanilide 4-hydroxylase (AcA hydroxylase). No, or only a very moderate, increase in the activity of aryl hydrocarbon hydroxylase was seen after OCS and HCB, respectively. Pretreatments with phenobarbital (PB) or 3-methylcholanthrene (MC) both increased AcA hydroxylase activity to a similar degree, whereas pretreatment with polychlorinated biphenyls (Aroclor 1254) had an effect equal to the sum of PB and MC. Judged from sodium dodecylsulfate polyacrylamide gel electrophoresis studies, OCS and HCB predominantly increased a microsomal polypeptide of apparent mol. wt 52,000, similar to PB. A reduced response was seen after OCS or HCB treatment of aromatic hydrocarbon 'non-responsive' DBA/2 (D2) mice compared to B6 mice, both with respect to AcA hydroxylase as well as EM demethylase and PNA demethylase activities. OCS treatment of B6D2F1 mice resulted in a doubling of AcA hydroxylase activity, but in mice of the (B6D2)D2 backcross no distinct subgroupings of individual AcA hydroxylase activities were apparent. These results demonstrate that OCS is an inducer of the PB-type in mice and that induction of AcA hydroxylase by OCS is not regulated by the *Ah* locus.

Octachlorostyrene (OCS; Fig. 1) is a byproduct in the manufacture of many chlorinated hydrocarbons. Together with hexachlorobenzene (HCB)*, OCS has been found to be a major organochlorine contaminant in fish caught in the Grenlandfjord region in Norway [1, 2] and in the Great Lakes in the U.S.A. [3].

Like HCB, a structurally related chlorinated hydrocarbon with a demonstrated carcinogenic effect in laboratory animals [4, 5], OCS has been shown to cause liver hypertrophy, megalocytosis, porphyria, increased heme biosynthesis as well as induction of liver microsomal cytochrome P-450 and associated monooxygenases in rats [6, 7].

Several reports have stated that HCB is a mixed type of inducer, giving a pattern of induced microsomal enzyme activities similar to the sum of the effects of PB- and MC-type of inducers [8–10]. In a recent work [7] we found that intraperitoneal or dietary administration of OCS or HCS to rats of both sexes seemed to increase primarily the PB-types of the cytochromes.

AcA has been used by Guenthner and Nebert [11] as a selective substrate for cytochrome P-448 metabolism. However, PB-treatment has also been found to increase AcA hydroxylase activity [7]. Therefore, it was not possible to decide whether the increase observed in the AcA hydroxylase activity after OCS- and HCB-treatment was due to an increase in a MC-type or PB-type of cytochrome. Studies with crosses and backcrosses of mice showing genetic differences in aromatic hydrocarbon responsiveness (for review see Ref. 12) might answer this question. It was therefore of interest to characterize the effects of OCS on the cytochrome P-450 system in such mice.

MATERIALS AND METHODS

Chemicals. OCS was a generous gift from Norsk Hydro (Porsgrunn, Norway). The compound was at least 98% pure as judged by gas chromatography on a Varian 1400 instrument equipped with the electrochemical detector. [^{14}C]Acetanilide (uniformly ring-labelled, sp. act. 10.5 mCi/mmole) was purchased from California Bionuclear Corporation

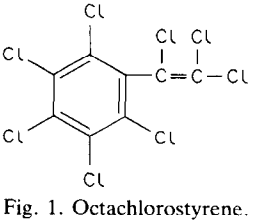

Fig. 1. Octachlorostyrene.

* Abbreviations used: PCB, polychlorinated biphenyls (Aroclor 1254); HCB, hexachlorobenzene; OCS, octachlorostyrene; PB, phenobarbital; MC, 3-methylcholanthrene; cytochrome P-450, a collective term for all forms of the cytochromes P-450; EM, ethylmorphine; PNA, 4-nitroanisole; AcA, acetanilide; AHH, aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase; B6, the inbred C57BL/6J mouse strain; D2, the inbred DBA/2J mouse strain.

(Sun Valley, CA) and [^{3}H]-4-hydroxyacetanilide (generally labelled, sp. act. 5.6 Ci/mmole) was purchased from New England Nuclear (Boston, MA). Other chemicals were obtained from the following sources: HCB (98% pure), Merck-Schuchardt (Darmstadt, F.R.G.); Aroclor 1254, Chem. Service (West Chester, PA); MC from Ferak (Berlin, F.R.G.); PB, AcA, EM and 4-hydroxyacetanilide (paracetamol) from the Norwegian Medicinal Depot (Oslo, Norway) and PNA, paranitrophenol, cytochrome *c*, NADP, NADPH and yeast glucose-6-phosphate dehydrogenase from Sigma Chemical Company (St. Louis, MO). Other chemicals were of reagent grade.

Animals and pretreatments. Male mice (20–30 g) were obtained from the following sources: specified pathogen free C57BL/6J (B6), DBA/2J (D2) and B6D2F1 from Bomholtgård Breeding and Research Centre, Denmark; (B6D2)D2 (backcross) was a generous gift from Dr. S. S. Thorgeirsson, National Institutes of Health (Bethesda, MD).

The animals were housed in plastic cages on hardwood bedding and were given a pelleted laboratory diet (Norwegian Standard) and water *ad lib.* Groups of mice were pretreated in the following way: single injections of various doses of OCS and HCB in corn oil intraperitoneally for up to 5 days before killing, 75 mg/kg PB in saline intraperitoneally 72, 48 and 24 hr before killing, 80 mg/kg MC in corn oil intraperitoneally 40 hr before killing or 500 mg/kg PCB in corn oil intraperitoneally 5 days before killing. Controls received vehicles only.

Preparation of microsomes. Livers from groups 10–15 mice were pooled and homogenized in 2 vol. of ice-cold 20 mM Tris buffer, pH 7.4, containing 1.15% KCl. In other experiments livers of individual mice were homogenized. Microsomal fractions were prepared as previously described [13] and stored in 30% glycerol–Tris–KCl buffer, pH 7.4, at −70° until use.

Microsomal assay. Cytochrome P-450 and cytochrome b_5 content of microsomes were measured by the method of Omura and Sato [14]. The activity of NADPH cytochrome P-450 reductase was measured by the rate of reduction of cytochrome *c* [15]. The EM *N*-demethylase assay is based on the formation of formaldehyde estimated according to Nash [16] using incubation conditions described previously [13]. The rate of oxidative *O*-demethylation of PNA was measured by spectrophotometric determination of 4-nitrophenol [17, 18] using the same incubation conditions as for the EM *N*-demethylase assay. Determination of AcA 4-hydroxylase using the high-pressure liquid chromatographic method of Guenthner *et al.* [19], was performed with [^{14}C]acetanilide and [^{3}H]hydroxyacetanilide using a Dupont Model 830 instrument equipped with a Dupont Zorbax ODS column eluted with methanol: H_2O (25:75). The activity of AHH was determined fluorometrically using benzo[*a*]pyrene as substrate by the method of Nebert and Gielen [20] as described [21] and expressed as pmole 3-hydroxybenzo[*a*]pyrene formed/mg microsomal protein per min. Protein concentrations were determined according to Lowry *et al.* [22] using bovine serum as standard.

Sodium dodecylsulfate polyacrylamide gel electrophoresis. Electrophoresis of mouse liver microsomes was performed on slab gels according to Laemmli [23] as described by Atlas *et al.* [24]. Each well was loaded with 12 μg protein. Ovalbumin (43,000), glutamate dehydrogenase (53,000), catalase (58,000) and bovine serum albumin (68,000) were used as standards. The gels were stained for protein and scanned at 570 nm with a Gilford scanning densitometer.

Statistics. All statistical comparisons were carried out using Student's *t*-test, and P values less than 0.05 were considered statistically significant.

RESULTS

The effects of a single intraperitoneal treatment of various doses of OCS and HCB on cytochrome P-450 and associated monooxygenase activities in the genetically polycyclic aromatic hydrocarbon 'responsive' B6 mice were compared (Fig. 2). A significant increase of EM *N*-demethylase, PNA *O*-demethylase and AcA hydroxylase activity was observed with both OCS and HCB beginning at doses of 10–50 mg/kg, with a maximal 2.2–4.0 fold induction at doses of 200 mg/kg. At doses of 100–200 mg/kg OCS or HCB, an increase (1.5–2.0 fold) in the content of cytochrome P-450 and the activity of cytochrome P-450 reductase were also seen. However, the activity of AHH was not increased by OCS, only a minor increase could be seen after the highest dose of HCB.

The time-course of the inducing effects of 200 mg/kg OCS and HCB was further examined (Fig. 3). Increases in the inducible monooxygenase activities were seen already after one day of treatment, whereas increases in cytochrome P-450 and cytochrome reductase became apparent first on the second day. Maxima were reached after 3–5 days.

The inducing effects of OCS and HCB on microsomal enzymes isolated from B6 mice were compared to the model inducers PB, MC and PCB (Table 1). Like PB, but contrary to MC, both OCS and HCB induced EM *N*-demethylase whereas no (OSC), or only a small increase (HCB), in AHH activity was seen. Further, no hypsochromic shift of the reduced CO-spectrum of cytochrome P-450 could be demonstrated. The magnitude of the increases caused by OCS and HCB was in the same order as that caused by PB. As expected with a 'mixed-type' of inducer, PCB induced EM *N*-demethylase activity similar to PB, AHH activity similar to MC and increased the level of cytochrome P-450 similar to the sum of PB and MC treatment. Noteworthy is that the increase in activity of AcA hydroxylase after PCB was equal to the sum of PB and MC treatment.

The data in Table 1 further show that the effects of the various inducers on liver cytochrome P-450 and associated monooxygenases isolated from the genetically polycyclic aromatic hydrocarbon 'non-responsive' D2 mice. As expected, no increases in any of the microsomal parameters is seen after MC-treatment, while the induction pattern following PB-treatment is comparable to that seen with B6 mice. As with MC, PCB did not induce AHH activity in the D2 mice. In contrast to PB, PCB gave a

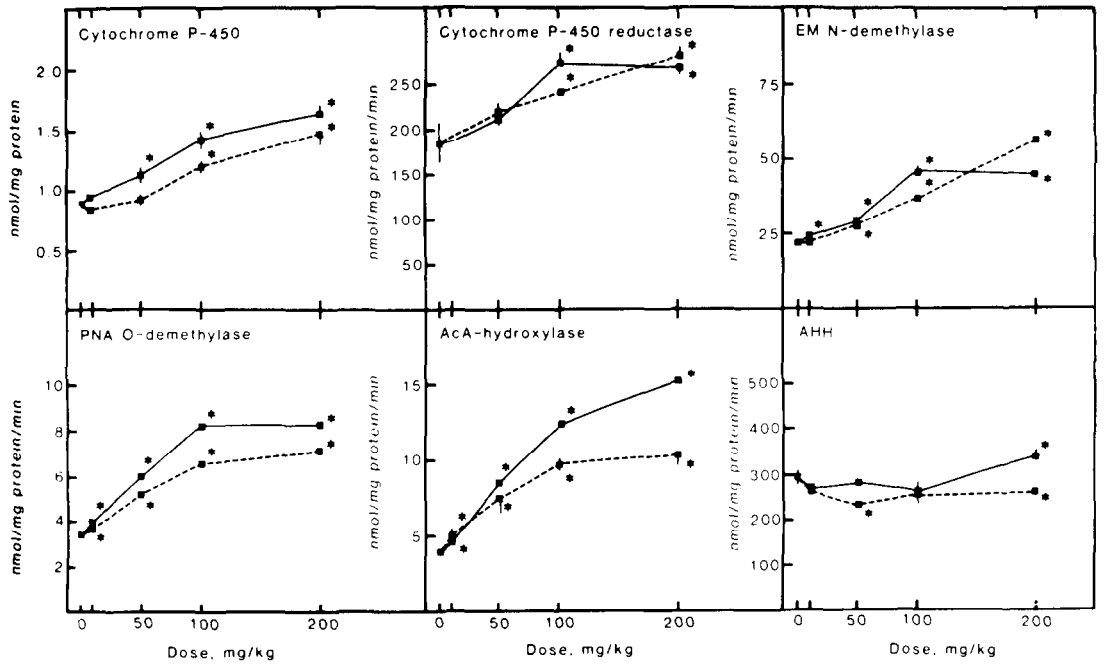

Fig. 2. Dose-dependency of the inducing effects of octachlorostyrene and hexachlorobenzene in B6 mice. Treatment consisted of various doses of OCS (■---■) or HCB (■——■) i.p. for 5 days. Values are means ± S.D. of three determinations with pooled fractions from 10–15 mice. * Statistically different from control, $P < 0.05$.

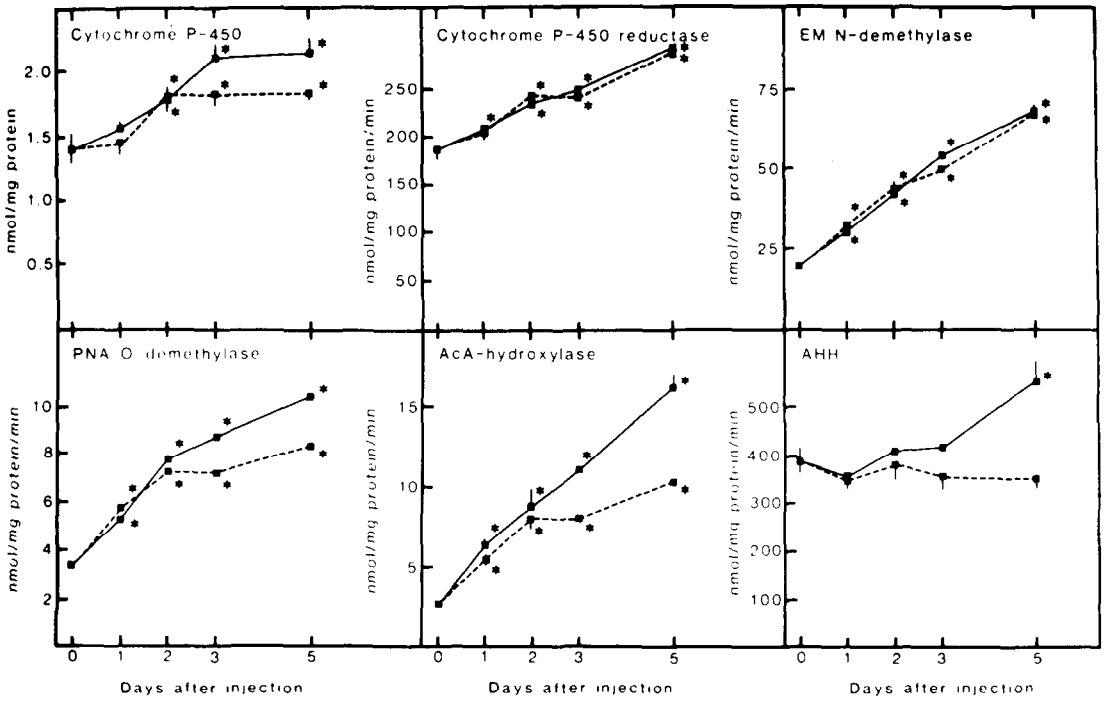

Fig. 3. Time-course of the inducing effects of octachlorostyrene and hexachlorobenzene in B6 mice. Treatment consisted of 200 mg/kg OCS (■---■) or HCB (■——■) i.p. for 1–5 days. Values are means ± S.D. of three determinations with pooled fractions from 10–15 mice. * Statistically different from control, $P < 0.05$.

Table 1. Comparison of the effects of OCB, HCB, PB, MC and PCB on various liver microsomal parameters in B6 and D2 mice

Assays	Control		OCS		HCB		PB		MC		PCB	
	B6	D2	B6	D2	B6	D2	B6	D2	B6	D2	B6	D2
Cytochrome P-450 (nmole/mg protein)	1.39 ± 0.12	1.24 ± 0.07	1.85 ± 0.03*	1.41 ± 0.14	2.13 ± 0.09*	1.43 ± 0.04	2.38 ± 0.09*	2.67 ± 0.02*	2.01 ± 0.14*	1.14 ± 0.05	2.91 ± 0.05*	1.60 ± 0.13*
Cytochrome b_5 (nmole/mg protein)	0.95 ± 0.04	1.01 ± 0.07	1.18 ± 0.02*	1.00 ± 0.06	1.29 ± 0.11*	1.10 ± 0.07	1.51 ± 0.06*	1.39 ± 0.12*	1.31 ± 0.02*	0.64 ± 0.06*	1.57 ± 0.03*	1.36 ± 0.33
Cytochrome P-450 reductase (nmole/mg protein/min)	184 ± 12	155 ± 2	285 ± 3*	190 ± 2*	293 ± 4*	180 ± 3*	290 ± 7*	303 ± 5*	212 ± 2*	157 ± 2	337 ± 4*	239 ± 10*
EM *N*-demethylase (nmole/mg protein/min)	19.0 ± 0.3	36.2 ± 0.8	67.5 ± 1.5*	53.2 ± 2.6*	67.9 ± 1.9*	48.0 ± 0.2*	89.9 ± 1.5*	107.8 ± 0.8*	16.9 ± 1.4	30.4 ± 1.8*	82.9 ± 1.7*	54.8 ± 0.8*
PNA *O*-demethylase (nmole/mg protein/min)	3.53 ± 0.06	5.01 ± 0.08	8.38 ± 0.05*	5.56 ± 0.60	10.52 ± 0.28*	7.0 ± 0.77*	10.44 ± 0.1*	8.36 ± 0.11*	6.84 ± 0.15*	4.91 ± 0.08	10.88 ± 0.18*	5.19 ± 0.05
AcA hydroxylase (nmole/mg protein/min)	2.72 ± 0.11	6.18 ± 0.23	10.44 ± 0.35*	7.57 ± 0.38*	16.57 ± 1.15*	7.49 ± 0.19*	12.42 ± 0.44*	10.60 ± 0.08*	12.79 ± 0.80*	3.99 ± 0.15*	25.01 ± 1.46*	6.53 ± 0.34
AHH (pmole/mg protein/min)	385 ± 38	281 ± 9	349 ± 21	281 ± 0	558 ± 61*	280 ± 22	509 ± 6*	303 ± 21	2250 ± 49*	251 ± 5*	1367 ± 30*	261 ± 12

Treatments consisted of 200 mg/kg OCS i.p. for 5 days, 200 mg/kg HCB i.p. for 5 days, 75 mg/kg PB i.p. daily for 3 days, 80 mg/kg MC i.p. for 40 hr or 500 mg/kg PCB i.p. for 5 days.
Values are means ± S.D. of three estimations with pooled fractions from 10–15 mice.
* Statistically different from controls, $P < 0.05$.

markedly lower response (1.0–1.5 fold) on cytochrome P-450 and on the other enzyme activities. This lowered induction response of PCB with D2 mice compared to B6 mice was also obtained after treatment with OCS and HCB.

Polyacrylamide gel electrophoresis has proved to be a useful technique for monitoring the relative amounts of individual liver microsomal membrane polypeptides after the administration of xenobiotics [25–27]. Gel electrophoresis of liver microsomes isolated from OCS treated B6 mice showed major increases in a band of apparent mol.wt of 52,000 and minor increases in band of mol.wt of 54,000 (Fig. 4). HCB-treatment resulted in a similar electrophoretic pattern, but in addition also minor increases in bands of mol.wt 51,000 and 55,000 were seen. Much like OCS and HCB, PB-treatment primarily increased a microsomal polypeptide of mol.wt 52,000. On the other hand, MC-treatment only increased a polypeptide of mol.wt 55,000, while PCB led to increases in polypeptides primarily of apparent mol.wt 52,000 and 55,000.

B6D2F1 and (B6D2)D2 mice were treated with OCS to test the possibility that OCS induction of AcA hydroxylase activity is regulated by the *Ah* locus. The results presented in Fig. 5 show that OCS-treatment of B6D2F1 mice induced cytochrome P-450 and associated monooxygenase activities to a lesser degree (1.0–1.7 fold) than in B6 mice, but comparable to that observed with D2 mice. OCS-treatment of (B6D2)D2 mice (Fig. 6) resulted in a broader range of individual values than in B6D2F1 mice, however no distinct subgroupings were apparent.

DISCUSSION

In certain areas, OCS has been found to be a major organochlorine contaminant in fish [1, 3]. OCS has structural similaries to HCB, and has been found to have many similar biological effects [6, 7]. Since HCB has been demonstrated to be carcinogenic both in hamsters [4] and mice [5], it is of considerable importance to characterize the long-term effects of OCS.

OCS and HCB treatment of B6 mice led to a time- and dose-dependent increase both in the levels of cytochrome P-450 and cytochrome b_5, and in the activities of cytochrome P-450 reductase, EM *N*-demethylase, PNA *O*-demethylase and AcA hydroxylase. Judging from the polyacrylamide gel electrophoresis studies, OCS and HCB predominantly increased a band of mol. wt 52,000. These results show that OCS and HCB under the present experimental conditions must be considered as inducers of the PB-type in mice similar to the results obtained in the rat [7]. However, HCB-treatment also gave a moderate increase in AHH activity at high doses. This finding, taken together with the demonstration that HCB-treatment also led to a small increase in a polypeptide of mol.wt 55,000 (MC-type), could reflect that HCB under certain experimental conditions could be a mixed-type of inducer, as claimed by others [8–10]. Another explanation could be that the MC-like part of the HCB-induction is depending on the degree of contami-

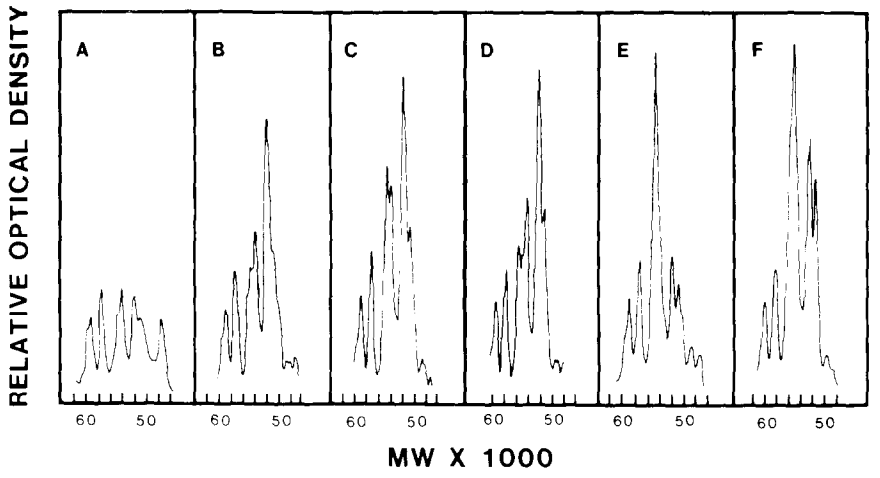

Fig. 4. Densitometric scannings of electrophoretograms of liver microsomes from control, OCS, HCB, PB, MC and PCB-pretreated B6 mice. Microsomal protein (12 μg per track) from B6 mice pretreated with various inducers, or a mixture of molecular weight standards were separated on 1.5 mm thick slab gels and stained with Coomassie brilliant blue® as described in Materials and Methods. (A) Control, vehicles only; (B) OCS-pretreated; (C) HCB-pretreated; (D) PB-pretreated; (E) MC-pretreated; (F) PCB-pretreated.

nations with very potent MC-like inducers, such as chlorinated dibenzo-*p*-dioxins and dibenzofurans.

The murine *Ah* locus is known to control the induction of cytochrome P_1-450, cytochrome P-448 and at least twenty associated monooxygenase activities, UDP-glucuronyltransferase activity as well as two cytosolic enzymes (reviewed in Refs. 12 and 28). Numerous conditions in the mouse, which include some types of chemically induced cancers, genotoxicity, cellular toxicity and birth defects, have been shown to be associated with the *Ah* locus. Even the simplest genetic model to explain the data on the regulation of aromatic hydrocarbon responsiveness involves several alleles and more than one locus (reviewed in Ref. 29). In studies on association of the *Ah* locus with various biological parameters, the use of offspring from appropriate crosses between B6 and D2 mice is ideal because the expression of AHH induction after MC-pretreatment is connected with a single gene difference: Ah^b (the allele in B6 mice) is the dominant allele for responsiveness, while Ah^d (the allele in D2 mice) is the recessive allele

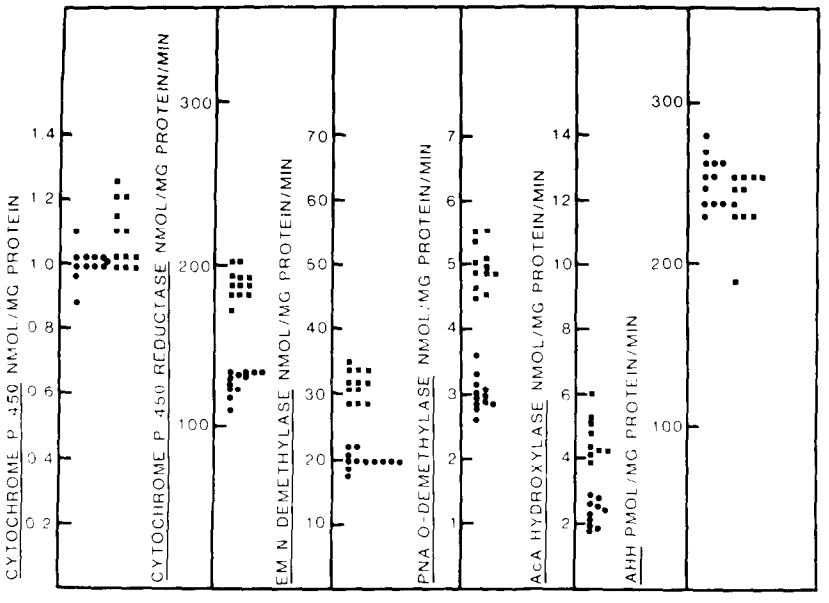

Fig. 5. Inducing effects of octachlorostyrene in B6D2F1 mice. Twelve mice were treated with 200 mg/kg OCS i.p. for 5 days (■) whereas the twelve control mice received vehicle alone (●). Microsomal assays were performed as described in Materials and Methods. Values are means of two determinations with liver microsomes isolated from individual mice.

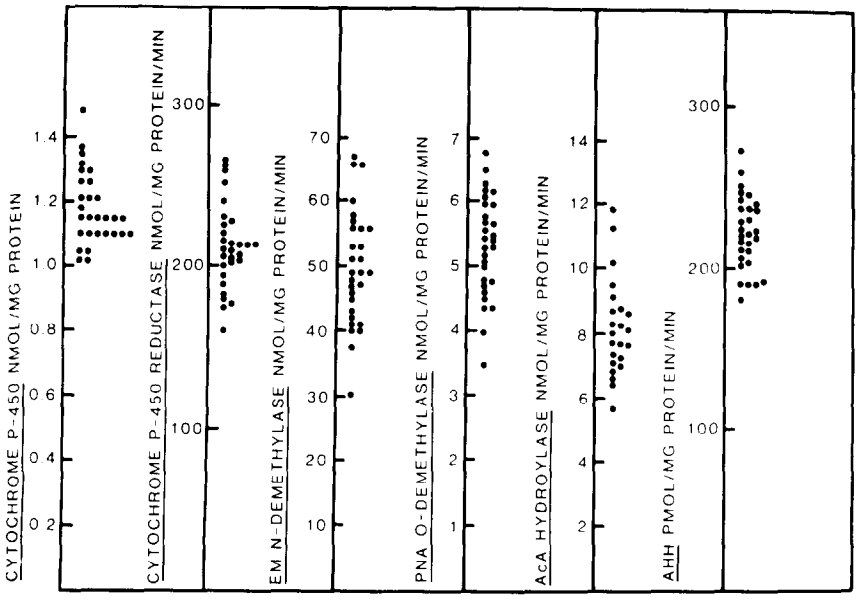

Fig. 6. Inducing effects of octachlorostyrene in (B6D2)D2 mice. Twenty-four mice were treated with 200 mg/kg OCS i.p. for 5 days (●). Microsomes were prepared and microsomal assays were performed as described in Materials and Methods. Values are means of two determinations with liver microsomes from individual mice.

[12]. Several studies have indicated that an important product of the *Ah* (regulatory) gene locus in mice is a cytosolic receptor, capable of binding certain polycyclic aromatic inducers (reviewed in Refs. 30 and 31). The inducer–receptor complex, after translocation into the cell nucleus, will then in some manner activate a group of structural genes [32]. Halogenated hydrocarbon inducers, such as TCDD are also able to interact with this receptor (reviewed in Ref. 30).

Since it has been claimed that AcA is a selective substrate for liver microsomal cytochrome P-448 [11], the present study employed B6 and D2 mice as well as their appropriate crosses to see whether an induction of AcA by OCS was mediated by the *Ah* locus. It was first demonstrated that AcA hydroxylase activity could be induced by OCS as well as PB and MC in responsive B6 mice, similar to what was seen in the rat [7]. Then it was shown that AcA hydroxylase activities were minimally altered by OCS in the non-responsive D2 mice, suggesting an involvement of the *Ah* locus. However, AcA hydroxylase activities were only doubled in B6D2F1 mice after OCS treatment compared to a 2.8–3.8 fold increase in B6 mice. Further, in individual (B6D2)D2 mice no distinct subgroupings were discernible after OCS treatment. This demonstrates that the OCS induction of AcA hydroxylase activity is not mediated by the *Ah* locus, but is probably catalysed by a PB-type of cytochrome P-450. This conclusion is also supported by the fact that the AcA hydroxylase activity was additively increased by the mixed-type inducer PCB in B6 mice.

In the genetically aromatic hydrocarbon 'non-responsive' D2 mice, OCS and HCB caused a lesser degree of induction compared to PB. A moderate degree of induction was also seen after OCS treatment of B6D2F1 mice. Recently, evidence has been presented showing that the induction of liver microsomal cytochrome P-450 (PB-type) and associated monooxygenases may involve a receptor which binds the inducer [33]. From the present study, however, it is not possible to conclude whether OCS uses such a PB-receptor or a different receptor in the induction process or whether OCS acts through a 'non-receptor' mechanism.

Acknowledgements—This study was supported by grants from the Norwegian Research Council on Science and the Humanities and N.A.T.O. Special Programme Panel on Eco-Sciences (No. SRG/13). We wish to thank Ms. Lise Timm Haug, Ms. Kristi Tveito and Ms. Ingrid Auglænd for technical assistance and Ms. Anna-Louise Midsem for preparation of the manuscript.

REFERENCES

1. G. Lunde and E. Baumann Ofstad, *Z. Analyt. Chem.* **282**, 395 (1976).
2. B. Bøe, *Norwegian Fish Directorate* No. 6/80, p. 1. Bergen (1980).
3. D. W. Kuehl, H. L. Kopperman and G. S. Weith, *Bull. envir. Contam. Tox.* **16**, 127 (1976).
4. J. R. P. Cabral, P. Shubic, T. Mollner and F. Raitano, *Nature, Lond.* **269**, 510 (1977).
5. J. R. P. Cabral, T. Mollner, F. Raitano and P. Shubic, *Int. J. Cancer.* **23**, 47 (1979).
6. J. J. T. W. A. Strik and J. H. Koeman, *Porphyrin in Human Diseases*, p. 418. Karger, Basel (1976).
7. J. A. Holme and E. Dybing, *Acta Pharmac. Tox.*, **50**, 41 (1982).
8. M. D. Stonard, *Biochem. Pharmac.* **24**, 1959 (1975).
9. J. A. Goldstein, M. Friesen, T. M. Scotti, P. Hickman, J. R. Hass and H. Bergman, *Tox. appl. Pharmac.* **46**, 633 (1978).

10. W. Vizethum, F. Burkhardt and G. Goerz, *Xenobiotica* **10**, 145 (1980).
11. T. M. Guenthner and D. W. Nebert, *Eur. J. Biochem.* **91**, 449 (1978).
12. D. W. Nebert and N. M. Jensen, *Crit. Rev. Biochem.* **6**, 401 (1979).
13. E. Dybing and T. Aune, *Acta Pharmac. Tox.* **40**, 575 (1977).
14. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
15. C. H. Williams and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
16. T. Nash, *Biochem. J.* **55**, 416 (1953).
17. A. H. Conney and J. J. Burns, *Adv. Pharmac.* **1**, 31 (1962).
18. K. Minck, R. R. Schupp, H. P. A. Illing, G. F. Kahl and K. J. Netter, *Archs Pharmac.* **279**, 348 (1973).
19. T. M. Guenthner, M. Negishi and D. W. Nebert, *Analyt. Biochem.* **96**, 201 (1979).
20. D. W. Nebert and J. E. Gielen, *Fedn. Proc.* **31**, 1315 (1972).
21. T. Aune, E. Dybing, S. D. Nelson and S. S. Thorgeirsson, *Acta Pharmacol. Tox.* **48**, 118 (1981).
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
23. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
24. S. A. Atlas, A. R. Boobis, J. S. Felton, S. S. Thorgeirsson and D. W. Nebert, *J. biol. Chem.* **252**, 4712 (1977).
25. D. E. Ryan, P. E. Thomas, D. Korzeniowski and W. Levin, *J. biol. Chem.* **254**, 1365 (1979).
26. R. N. Sharma, R. G. Cameron, E. Farber, M. J. Griffin, J. Jolly and R. R. Murray, *Biochem. J.* **182**, 317 (1979).
27. E. F. Johnson, in *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. J.-Å. Gustavsson, J. Carlstedt-Duke, A. Mode and J. Rafter), p. 65. Elsevier/North-Holland Biomedical Press, Amsterdam (1980).
28. D. W. Nebert, S. A. Atlas, T. M. Guenthner and R. E. Kouri, in *Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment* (Eds. P. O. P. Ts'o and H. V. Gelboin), p. 345. Academic Press, New York (1978).
29. D. W. Nebert, J. R. Robinson, A. Niwa, K. Kumaki and A. P. Poland, *J. cell. Physiol.* **83**, 393 (1975).
30. A. Poland and A. Kende, in *Origins of Human Cancer* (Eds. H. H. Hiatt, J. D. Watson and J. A. Winsten), p. 847. Cold Spring Harbor Laboratory (1977).
31. A. Poland and E. Glover, *J. biol. Chem.* **251**, 4936 (1976).
32. A. B. Okey, G. T. Bondy, M. E. Mason, G. F. Kahl, H. J. Fasen, T. M. Guenthner and D. W. Nebert, *J. biol. Chem.* **254**, 11636 (1979).
33. A. Poland, I. Mak, E. Glover, R. J. Boatman, F. H. Ebetino and A. S. Kende, *Molec. Pharmac.* **18**, 571 (1980).