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2,3,4,4′,5-Pentachlorobiphenyl: differential effects on C57BL/6J and DBA/2J inbred mice

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Polychlorinated biphenyls (PCBs) typify a group of halogenated aromatic chemicals which include the polychlorinated dibenzofurans (PCDFs), dibenzo-*p*-dioxins (PCDDs), naphthalenes and polybrominated biphenyls (PBBs). These chemicals have a number of common chemical and biological properties [1], e.g. (1) within each group there exists a multiplicity of isomers and congeners, (2) there are dramatic effects of structure on the biologic and toxic potencies of individual halogenated aromatic compounds, and (3) like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic individual halogenated aromatic compound, a limited number of approximate stereoisomers elicit comparable toxic responses [2–9]. Included in this latter group are 3,3′,4,4′,5,5′-hexachlorobiphenyl (HCBP) and hexabromobiphenyl (HBBP) [2, 9]. In addition, it has been shown that the 2,3,7,8-TCDD and related compounds induce the microsomal cytochrome P-450-dependent monooxygenase, benzo[*a*]pyrene hydroxylase (or aryl hydrocarbon hydroxylase, AHH) and reversibly bind to a high-affinity cytosolic receptor protein [3, 10, 11]. The non-covalently bound ligand (inducer)–receptor complex is transported into the nucleus and subsequently controls the expression of AHH induction and related toxic responses [9–13]. The cytosolic receptor protein, a product of the *Ah* locus in mice, is found in higher concentrations in certain mouse strains, such as the C57BL/6J inbred strain [12, 13], which are highly sensitive to the biologic and toxic effects of 2,3,7,8-TCDD, 3,3′,4,4′,5,5′-HCBP, 3,3′,4,4′,5,5′-HBBP and related compounds [2, 9, 14]. In contrast, the DBA/2J mouse strain contains lower levels of the high-affinity receptor protein and is much less susceptible to the inductive and toxic effects of 2,3,7,8-TCDD and related compounds [9, 14].

Recent reports have described a group of PCB and PBB isomers and congeners that induce a pattern of hepatic microsomal enzyme activities consistent with the coadministration of 3-methylcholanthrene (3-MC, an AHH inducer) plus phenobarbitone (PB) [8, 15–19]. Some of these mixed-type inducers are toxic to chick embryos [20] and rats [21]; however, their toxicities and activities as AHH inducers in inbred mouse strains have not been determined. This study reports the biologic and toxic effects elicited by 2,3,4,4′,5-pentachlorobiphenyl (PCBP), a mixed-type inducer in rats, in the responsive C57BL/6J and the non-responsive DBA/2J mice.

PCBP and 2,2′,4,4′,5,5′-HCBP were > 99% pure and prepared as described [15]. The synthetic route and purification procedures were developed to preclude the presence of PCDD and PCDF contaminants. Ascorbic acid and phenazine ethosulfate were purchased from the Sigma Chemical Co., St. Louis, MO. The sources of other chemicals used in this study are described elsewhere [15]. Adult C57BL/6J and DBA/2J inbred strains of mice were obtained from the Jackson Laboratory, Bar Harbor, ME. The mice were housed in wire cages, maintained on a 12-hr diurnal light regimen, and allowed free access to Purina Certified Rodent Chow (No. 5002) and water. Both strains of mice were injected intraperitoneally with PB or 3-MC (400 μmoles/kg body wt) on three consecutive days, and the animals were killed by cervical dislocation 24 hr after the last injection. The C57BL/6J mice were injected with two doses of PCBP (either 150 or 750 μmoles/kg) on days 1 and 3 and killed on day 6. DBA/2J mice received only the high dose of PCBP. Mice injected with corn oil (20 ml/kg) serves as controls. All animals were fasted over the last 24 hr to lower liver glycogen levels. The thymic involution studies were carried out as described above except that the animals were not fasted over the last 24 hr.

The mouse livers were cleared of blood by perfusing via the hepatic portal vein with ice-cold isotonic saline containing EDTA (100 μM), and the liver weights were determined. Thymuses were excised, trimmed, blotted dry, and weighed. The liver microsomal fraction was isolated as a 100,000 *g* pellet by further centrifugation of a 10,000 *g* supernatant fraction from the whole liver homogenate [8].

The concentration of cytochrome P-450 was determined

Table 1. Effects of 3-MC, PB and PCBP as inducers of drug-metabolizing enzymes in C57BL/6J and DBA/2J inbred strains of mice

Treatment	Strain (N)	Liver wt (% of body wt)	Cytochrome P-450		NADPH–cytochrome c reductase*	DMAP *N*-demethylase*	Aryl hydrocarbon hydroxylase†
			nmoles/mg protein	λ_{max} (nm)			
Corn oil	C57BL/6J (8)	5.24 ± 0.60	0.522 ± 0.053	450.0	154 ± 21	7.91 ± 1.11	245 ± 28
	DBA/2J (5)	5.93 ± 0.93	0.503 ± 0.021	450.0	142 ± 21	4.70 ± 1.07	216 ± 18
PB	C57BL/6J (6)	6.83 ± 1.17‡	1.21 ± 0.11‡	450.0	306 ± 16‡	22.9 ± 2.8‡	466 ± 25
	DBA/2J (4)	7.77 ± 1.18‡	1.43 ± 0.21‡	450.0	295 ± 25‡	21.9 ± 2.9‡	448 ± 34‡
3-MC	C57BL/6J (7)	5.82 ± 0.65	1.29 ± 0.07‡	448.0	165 ± 12	10.3 ± 1.7	3990 ± 600‡
	DBA/2J (5)	4.99 ± 0.33	0.484 ± 0.030	450.0	136 ± 11	5.93 ± 1.23	243 ± 10
PCBP§ (150 μmoles/kg)	C57BL/6J (3)	5.25 ± 0.22	0.994 ± 0.067‡	449.0	182 ± 9	9.90 ± 1.71	2400 ± 625‡
PCBP (750 μmoles/kg)	C57BL/6J (3)	8.12 ± 2.13‡	1.84 ± 0.06‡	448.5	235 ± 13‡	16.4 ± 3.4‡	4160 ± 210‡
PCBP (750 μmoles/kg)	DBA/2J (3)	5.07 ± 0.40	0.774 ± 0.081‡	450.0	255 ± 16‡	13.9 ± 0.8‡	198 ± 17

* Expressed in nmoles·(mg protein)$^{-1}$·min^{-1}.
† Expressed in pmoles·(mg protein)$^{-1}$·min^{-1}.
‡ Statistically different from corn oil controls (same strain) at the 1% level of significance.
§ Administered on days 1 and 3; the animals were killed on day 6.

Table 2. Effects of PCBs on thymus weight in the inbred strains of mice

Treatment* (dose)	Strain	Thymus wt (% of body wt)
Corn oil	C57BL/6J	0.20 ± 0.07
PCBP (150 μmoles/kg)	C57BL/6J	0.19 ± 0.06
PCBP (750 μmoleskg)	C57BL/6J	0.12 ± 0.04†
PCBP (1500 μmoles/kg)	C57BL/6J	0.10 ± 0.04†
2,2′,4,4′,5,5′-Hexachlorobiphenyl (1500 μmoles/kg)	C57BL/6J	0.33 ± 0.10†
Corn oil	DBA/2J	0.22 ± 0.06
PCBP (1500 μmoles/kg)	DBA/2J	0.25 ± 0.04
2,2′,4,4′,5,5′-Hexachlorobiphenyl (1500 μmoles/kg)	DBA/2J	0.26 ± 0.04

* PCBs or corn oil were injected on days 1 and 3 and the animals were killed on day 6.

† Statistically different from corn oil controls (same strain) at the 1% level of significance. N = 8.

by the method of Johannesen and DePierre [22]. This method was used to compensate for both hemoglobin and methemoglobin which heavily contaminated those microsomes prepared from poorly-perfused livers. Briefly, methemoglobin was reduced to hemoglobin by adding ascorbic acid (250 μM) and phenazine ethosulfate (2.5 μM), following which cytochrome P-450 was determined from the difference spectrum of dithionite-reduced microsomes minus dithionite-free microsomes with carbon monoxide present in both the sample and reference cuvettes. The activities of 4-dimethylaminoantipyrine (DMAP) *N*-demethylase [23], AHH [24] and NADPH–cytochrome *c* reductase [25] were measured as described, with minor procedural modifications [15]. Protein concentration was determined by the method of Lowry *et al.* [26]. The final concentration of microsomal protein was 100 μg/ml in all other assays.

Results are presented as means ± standard deviation. The number of animals per group is given in the tables of results. Statistical significance was analyzed by Dunnett's method for multiple comparisons with a control [27].

The effects of PCBP as an inducer of hepatic microsomal enzymes in C57BL/6J and DBA/2J mice are summarized in Table 1. In the responsive mice, there was a dose-dependent induction of AHH activity and a downfield shift of the reduced cytochrome P-450:CO difference spectrum absorption maximum from 450 nm (for the control animals) to 448.5 nm for those mice treated with PCBP at 750 μmoles/kg. At a comparable dose of PCBP in the DBA/2J mice, the activity of AHH and the position of cytochrome P-450:CO difference spectrum peak were unchanged. In contrast, PCBP induced NADPH–cytochrome *c* reductase and DMAP *N*-demethylase in both inbred strains. Thus, the mixed-type pattern of microsomal enzyme induction observed in immature male Wistar rats [15] for PCBP was duplicated in the responsive C57BL/6J mice. However, like 3-MC, 3,3′,4,4′,5,5′-HCBP and HBBP [9, 14], administration of PCBP to the non-responsive DBA/2J mice did not result in the induction of microsomal AHH but did induce PB-type activity.

This lack of AHH inducibility in the DBA/2J mice supports the hypothesis that the *Ah* locus plays a major role in mediating the effects of the mixed-type inducer, PCBP, as well as 2,3,7,8-TCDD and related compounds.

Table 2 summarizes the effects of PCBP and a PB-type inducer, 2,2′,4,4′,5,5′-HCBP, on thymus weights in the responsive and the non-responsive mice. The former compound caused a dose-dependent reduction in thymus weights in the responsive C57BL/6J mice but did not affect this organ in the DBA/2J mice even after administration of 1500 μmoles/kg. 2,2′,4,4′,5,5′-HCBP did not decrease the thymus weights in either mouse strain. Thus, the activity of PCBP in causing thymic involution is qualitatively similar to that of 3,3′,4,4′,5,5′-HBBP and related toxic stereoisomers of 2,3,7,8-TCDD; however, no conclusion can be made about segregation with the *Ah* locus until the backcross experiments are carried out.

Previous reports have suggested that the activities of PCBs as AHH inducers are dependent on the coplanarity, substitution pattern, and polarizability of the lateral substituents [28, 29]. This last structural requirement stipulates the presence of four or more laterally-substituted chlorine atoms with at least two substituents on each ring. This hypothesis is not consistent with the reported AHH-inducing activity of several PCB congeners that contain only one laterally-substituted chloro group. These congeners are 3,4,4′,5-tetra-, 2,3,4,4′,5,6-hexa- and 2,3,4,4′,5-pentachlorobiphenyl [15, 16], and this report demonstrates the AHH-inducing activity of the latter congener in the C57BL/6J mice. Moreover, it has also been shown that, like TCDD and other 3-MC-type or mixed-type inducers, 3,4,4′,5-tetra- and 2,3,4,4′,5-pentachlorobiphenyl bind to a hepatic cytosolic receptor protein from immature male Wistar rats [30]. The precise structural requirements enabling PCBs and related halogenated aromatics to induce AHH activity and to bind to the receptor protein have not been fully delineated and are currently being investigated in our laboratory.

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Novel combination chemotherapy of experimental trypanosomiasis by using bleomycin and DL-α-difluoromethylornithine; reversal by polyamines*

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Previously we reported that an irreversible inhibitor of polyamine biosynthesis, DL-α-difluoromethylornithine (DFMO), cured mice infected with *Trypanosoma brucei brucei*, a parasite of game and cattle in Africa [1], and also cured similar infections of *Trypanosoma b. rhodesiense*, a human sleeping sickness pathogen [2]. DFMO is a specific inhibitor of ornithine decarboxylase (ODC), the major rate-controlling enzyme of polyamine biosynthesis [3–5], and slows tumor cell replication *in vitro* and *in vivo* [6, 7]. Trypanosome cures effected by DFMO can be blocked by coadministration of the commonly occurring polyamines putrescine, spermidine or spermine [2, 8]. We have found recently that another agent, the potent antitumor antibiotic Blenoxane (a commercial mixture of bleomycinic acid derivatives, hereafter referred to as bleomycin) also cures this infection [9]. Bleomycin inhibits nuclear division and causes malformation of the nucleus and disorders of microtubule morphology in *Trypanosoma b. gambiense* [10], another human sleeping-sickness pathogen. Cures by bleomycin can also be blocked by spermidine and spermine but not putrescine [9]. Thus, two agents which have antitumor properties also seem to restrict parasite growth in infected animals in a manner analogous to their antineoplastic effects.

Modern chemotherapies of neoplastic disease in fact routinely employ drugs in combination [11, 12]. Such regimens in some instances have proven significantly better for prolonging life and even obtaining permanent clinical remission than single-drug therapy [11, 12]. In contrast to the highly developed protocols for neoplastic disease, no such drug-combination regimens have been developed for chemotherapy of hemoflagellate infections. These rapidly growing organisms, including *Leishmania* spp. and *Trypanosoma* spp. cause several debilitating diseases such as visceral leishmaniasis, Chagas' disease and sleeping sickness, and therefore cause vast human suffering and economic loss in Asia, Africa and South America. New drugs or drug combinations are needed to supplant the toxic and often ineffective agents currently in use for these diseases [13–15]. We now report successful combination chemo-

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